Exploring Biofilm in Surgical Operation Theatres

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Abstract
Hygienic and aseptic conditions in medical and health facilities are a factor of high importance. Recently, some cases of post-surgery infections were noticed even with strict and professional disinfection protocols. This triggers the presumption of a hidden infection source, which may be due to the construction-reconstruction cycle of a biofilm. While this phenomenon is well studied and treated in some places in operating theatres, namely, equipment with water flow inside, the infections may be sourced from other parts or surfaces, which must be determined. In order to investigate, biofilm existence on surfaces in operating rooms was tested using the traditional crystal violet assay. After that, coupons were fixed in selected places in the same rooms to affirm and quantify biofilm existence. Finally, the source of pollution was investigated, confirming that it comes from cleaning water buckets, as one of the other less common sources. Results show that biofilms establish on various surfaces in surgical operating theaters as a result of errors in primary cleaning associated with the fact that disinfection protocols were targeting planktonic, not biofilm bacteria. This suggests rendering the current disinfection protocols to include biofilm.

Keywords: *Biofilm, Operating theatres, Surgical hygiene, Sterilizing protocol, medical pollution*

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1. Introduction

One of the greatest challenges facing medical facilities, especially surgical theatres, clinics and biological laboratories, is biofilms, which serve as covert homes for infectious and non-infectious microbes [1]. Communities of microbes known as biofilms adhere to surfaces and coat themselves with an extracellular polymeric substance (EPS) that defends them against harsh outside influences, such as antibiotics [2]. Such biofilms may develop on an array of surfaces throughout hospitals, including implants, surgical instruments, water lines, and even those surfaces that are thought to be sterile [3].

Most sterilization protocols aim to reduce or eliminate planktonic bacteria in both forms, airborne or waterborne, or attached to a surface before a biofilm can form [4]. However, if the sterilization protocols are at fault, biofilms may develop on tables, instruments used for surgery, and even “sterile” surfaces. For individuals, especially those with compromised immune systems, these biofilms may have serious effects of implant failures and ongoing infections [5]. Investigations have shown that the creation of biofilms has been linked to up to 65% of healthcare infections [6]. Avoiding the effects of biofilms at surgical sites (surgical-site infections, SSIs) and infections related to healthcare (healthcare acquired infections, HAIs) needs the biofilms in these kinds of environments and their sources to be found and identified.

However, since they remain hidden and resistant to traditional disinfection strategies, it is hard to find the source and reservoirs of infection. Consequently, to identify biofilms being formed in their initial phases, advanced methods including microscopy, molecular techniques, and biosensors are utilized, but their use is limited as such techniques are often implemented using exposure coupons and small samples and cannot be done in-situ; hence, a lack of representativity [7, 8].

Biofilms are produced by the interaction of colonies of microbes and extracellular polymers (EPS). This EPS is made up of sugars, proteins, and nucleic acids released by microbes throughout the growth period and irreversibly binds to the surface. A biofilm matrix may also include non-cellular materials such as metals, crystals, and corrosion molecules, mud or silt particles, or blood components depending on the conditions in which biofilms are formed [9, 10]. Microorganisms can attach to, and form biofilms on, surfaces that are biotic as well as abiotic [11]. Furthermore, it is believed that microbes may adapt to harsh environments by creating biofilms,

which is a protective development mode [12]. In the process of protecting microbial cells from damaging conditions, which include extreme temperatures, dehydration, starvation, and even antimicrobial medications or disinfection, the biofilm acts like an inhibitor that creates an environment that is ideal for microbial cell activity [13]. As a result of this, microorganisms can swiftly establish themselves, defend against attack, and enhance their attachment to the host surface to propagate disease as time passes. Thus, the first stage of defense for microorganisms is biofilm [14].

In recent investigations, different types of biofilms have been shown on medical instruments and in the water system of a manufacturing process. Complex biofilms consisting of filamentous microbes, aqueous diatoms, clay particles and corrosion-related substances were found in the water system, while the biofilms on surgical instruments consisted of only one coccoid organism and the extracellular polymer matrix (EPS) it produces [10].

In studying the cleaning in one establishment, it was noticed that the primary cleaning process used water and detergents that are not fully aseptic due to the belief that this initial cleaning would be followed by normal sterilizing protocols to kill every living contaminant. From the current knowledge about the advised steps for sterilization, this should hold true. However, from the biofilm science point of view, such a process may be a driving force for biofilm formation. That is, bacteria are spread by primary cleaning using water and cleaning detergents. After that, normal sterilizing protocols are applied which, if they fail to kill the bacteria, induce a stress which can drive the bacteria to form biofilms [15].

Biofilms can grow on a wide range of surfaces, such as natural hydro systems, industry or municipal water pipes, permanent medical equipment and facilities, and living tissue [16]. The theory suggests that biofilm is built on various surfaces in operating theatres, providing a reservoir of infection which can be transferred to wounds through human touch. In this study, we investigate if this theory explains the infections found and suggests ways to counter any problem.

The research consisted of three stages, first measuring the bacterial pollution in primary cleaning tools, namely, water buckets. Secondly, investigating the existence of biofilm on selected surfaces in operating rooms. Thirdly, making quantitative measurements of biofilm to determine the extent of the problem.

2. Materials and Methods

This research explores the existence of biofilm in operating rooms. The experiments include three levels of tests. First, a fast examination of the existence of biofilm on targeted surfaces using crystal violet assay [17]. Second is a quantitative estimation of biofilm growth on those surfaces using the viable count method [18]. The third is an examination of the bacterial content (cfu ml^{-1}) of the water in buckets used in primary cleaning before the daily aseptic sterilization.

2.1 Preparation for crystal violet assay

As this assay needs a contact time of more than 30 minutes, experiments were implemented on a horizontal surface. The other requirement is that, as the CV assay is a color analysis technique, it requires light-colored surfaces such as trollies, floors, walls, shelves and drawers to give the best results. It is hypothesized that other parts of the operating room with dark surfaces will develop biofilms in the same way, since the same cleaning and sterilizing procedure is followed. The CV assay is a primary test that should be confirmed by attaching biofilm coupons for biofilm viable count experiments.

2.2 Biofilm bacterial viable count assay

In order to estimate the quantity of biofilm developed on surfaces in the operating rooms the following procedure was followed. Coupons (standard microscope slides of 76 by 25 by 1 mm) were fixed with glue on selected surfaces and equipment. Cleaning and sterilizing teams were advised to follow their ordinary protocols on all facilities under study including these coupons. The coupons were exposed for 10 days. The technique used for this assay is that mentioned by Lewandowsky and Beyenal [18].

2.3 Biofilm optical density measurement

Biofilm growth rate was measured by optical density using a recently developed technique [19]. This technique could be summarized by putting a biofilm coupon between a light source and a light intensity measuring device. The more the biofilm exists, the less the light penetrates the coupon. This technique is an inclusive one and measures all the slide area that microscopy techniques cannot do. The used light is the visible “white” one with a wavelength of 380 nm to 750 nm.

2.4 Primary cleaning-water bacterial content

As it is hypothesized, biofilm formation can be triggered when polluted primary cleaning water is mixed with cleaning detergents. This step is magnified when bacteria are exposed to two kinds of stress: drought and biocides. Therefore, a heterotrophic plate count (HPC) of the primary cleaning water was made.

It was found that, during daily cleaning, water was changed after use in 2-3 operating rooms according to a visual estimate of dirtiness and not according to bacterial pollution. Samples were taken before and after every use in each operating room.

2.5 Frequency of samples

Every point on every set was the average of three samples. That is, for example, in every place of coupon

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attachment, three microscope slides were fixed for testing. Water samples were taken three times for every point and the average was calculated.

2.6 Catchment area

Operating rooms were selected arbitrarily in some hospitals in Iraq. They are numbered from 1 to 10. The name of both hospitals and operating rooms are requested to be hidden for administrative purposes.

2.7 Controls

In order to identify the extent of the problem of biofilm in surgical operating rooms, control slides were fixed in selected places along them. Cleaning workers were advised to avoid touching those slides or including them in any cleaning activities in order to compare them with the other slides under test. Three control slides were spread into three selected positions in every operating room. Every test in every test location was compared to those controls. The aim of controls is to investigate if there are any other factors that drive biofilms to form other than the cleaning misuse. Controls followed the same biofilm testing procedure.

3. Results

3.1 Crystal violet assay

Crystal Violet assay showed the presence of biofilm at all locations in the operating rooms

3.2 Biofilm bacterial viable count assay

Average bacterial colony counts (cfu cm^{-2}) at the selected site within the ten operating rooms are shown in Figure (1).

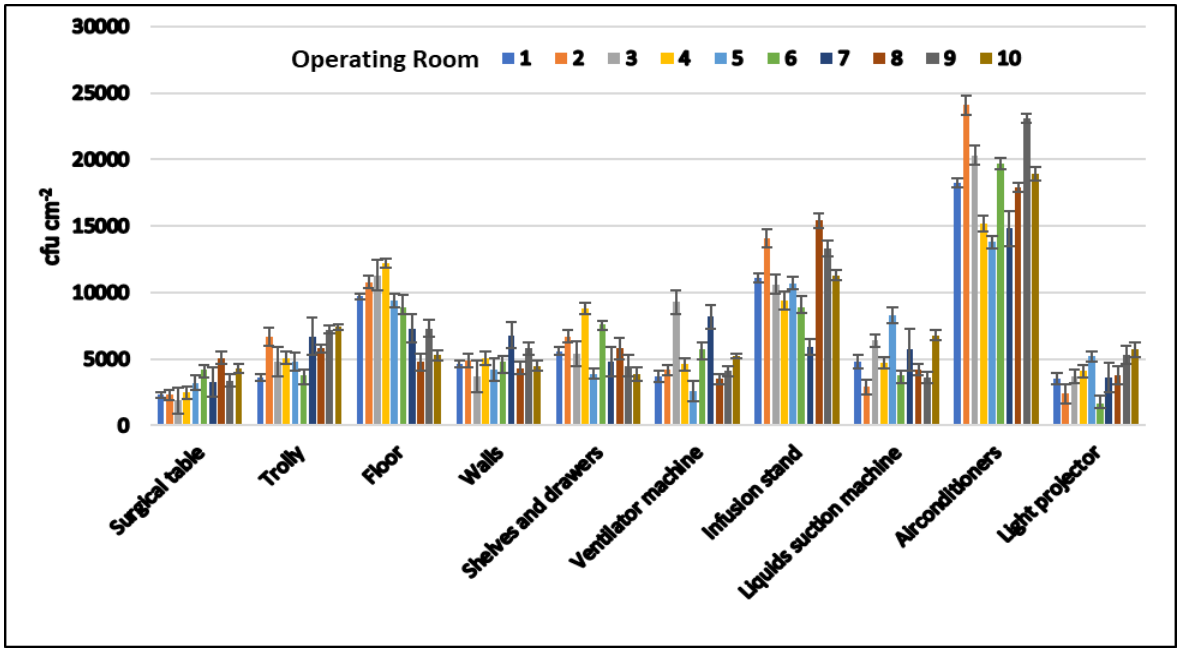

Figure (1): Biofilm viable count for 10 operating rooms and their equipment under study.

3.3 Biofilm optical density

Biofilm formation, as measured by optical density, is shown in Figure (2).

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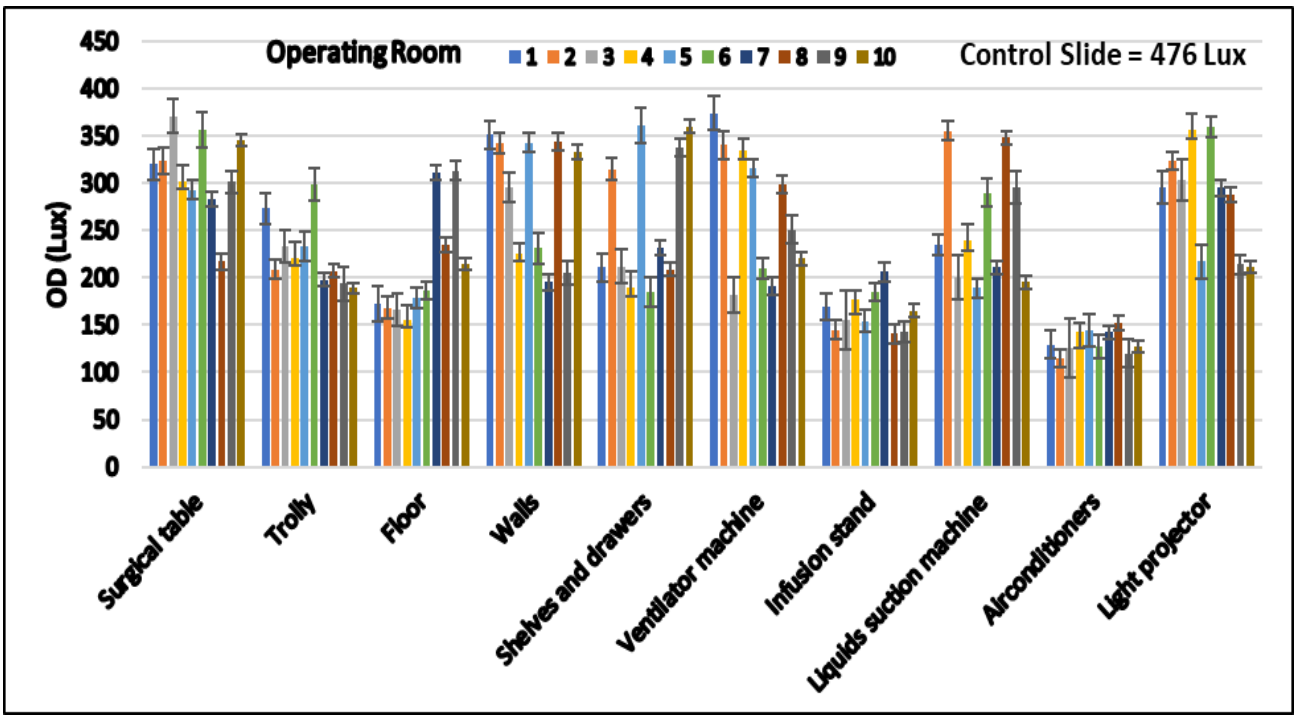

Figure (2): Biofilm optical density reading for 10 operating rooms and their equipment

3.3 Cleaning water buckets HPC

Results showed an increasing bacterial load after each room was cleaned. Figure (3).

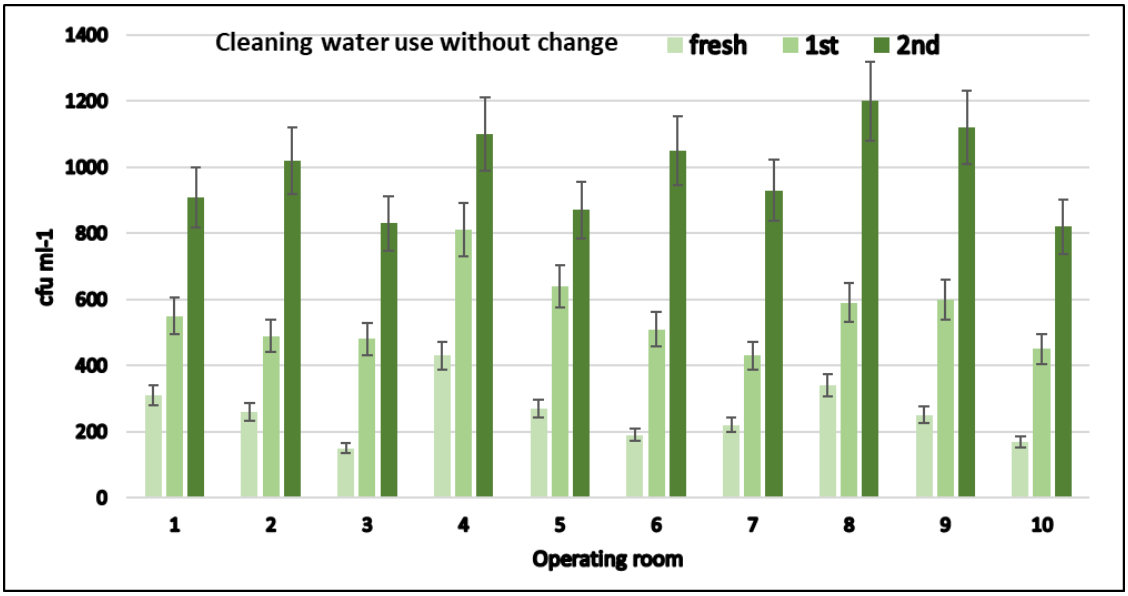

Figure (3): Bacterial count in the cleaning water bucket before (fresh), first, and second operating room cleaning

3.4 Microscope images

As a double check of biofilm formation on the studied sites, microscope images for sample slides were taken. Figure (4) shows the obtained images.

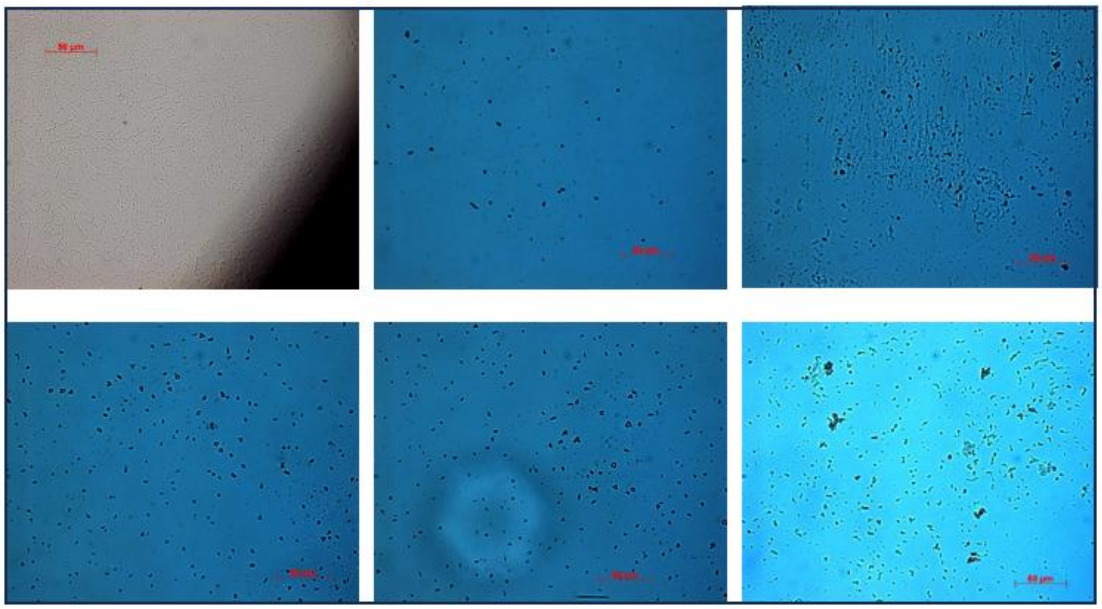

Figure (4): biofilm in some slides in operating room 1, upper left is control, upper middle is surgical table, upper right is trolly, lower left is shelves, lower middle is ventilator machine, lower right is light projector. Red bars are equal to 50 µm

4. Discussion

The theory behind this work is that any microorganisms on a surface exposed to a sterilization technique will either be killed or form a biofilm. The degree to which biofilms are formed rather than completely Sterilization depends on whether the bacteria on the surface are planktonic or already in a biofilm or if the disinfection process and dose are fully or partially lethal to planktonic bacteria. Table 1 summarizes those scenarios.

Table (1): Biofilm existence scenarios

Form of life / Biocide lethality	Planktonic	Biofilm
Fully lethal	No Biofilm [20]	Biofilm Increases [15]
Partially lethal	Biofilm develops [21]	Biofilm Increases [15]

Before going deep, “fully lethal” here represents the higher lethal concentration. For instance, Ethyl Alcohol full lethality is with a 70% mixture with water. More concentrations lead to less biocide effect. This because water denatures proteins in addition to its existence with Alcohol slows the evaporation; hence, more exposure time which is necessary to cell penetration [22, 23]. Moreover, 100% Alcohol could stimulate outer proteins to coagulate and form protective shell that push the cell content to be trapped inside [24].

Unless stringent measures are taken, the probability of avoiding biofilm development is low; Prevention requires there to be few microorganisms on the surfaces and low levels of planktonic bacteria coupled with high concentrations of fully lethal biocide. Bacteria can arrive at a surface either through the air, or, as shown in this work, during primary cleaning where the level of bacterial pollution in the used cleaning water buckets is not fully controlled. In the latter case, the level of disinfectant in the cleaning water is not fully lethal. Biofilm formation then occurs for three reasons, first the cleaning workers may lack training and cleaning detergents mixtures used or fail to maintain the correct level of detergents. Secondly, the professional staff rely on the currently followed sterilization protocol to be enough to remove microorganisms even if they have been spread by the primary cleaning step. Third, there is a lack of understanding of how biofilms develop in such scenarios. Most cleaning and sterilizing protocols that are implemented in operating rooms and elsewhere in medical and health establishments do not take the phenomenon of biofilm formation into consideration and, while the formation and effects of biofilms are well known in some medical areas such as dentistry and the prolonged use of catheters,و they are poorly understood by most staff.

To investigate the hypothesis, the study started with testing the existence of biofilm on selected surfaces in the operating rooms. These surfaces were necessarily horizontal and of white or light color in order for the in-situ crystal violet assay to work and be seen. All the selected surfaces in every operating room showed a bluish cloud of bacteria in biofilm after 30 minutes, easily seen by the naked eye.

These initial observations led to exploring biofilm attachment on other surfaces. Attaching microscope slides in-situ on selected surfaces enabled observation of potential biofilm formation in greater depth than the crystal violet assay.

When considering biofilm quantitative analysis, air conditioning units show that they may be an important cause of bacterial contamination by dispersing bacteria through the air circulation. Biofilm growth in such appliances is a well-known phenomenon, having all the requirements that bacteria need [25, 26]. Biofilm formation in air conditioners may have a positive effect in filtering and cleaning the air instead of being an infection source [27, 28]. However, while this has yet to be studied by examining bacterial concentrations in both in and out air streams. In our studies, it is normal practice to periodically treat the air-conditioning systems and filters using specialized disinfection procedures [29, 30].

When taking a look at Fig. 1, the trends also show that the biofilm tensity depends on two other factors, frequency of cleaning and biofilm essentials abundance. Surgical tables and even though they are most exposed to those two factors, they admit less biofilm. This is because those surgical rooms components are covered with suitable duvets after every surgery. On the other hand, those beds are exposed directly to various human body liquids, which represent a good driving force for biofilm formation. The obtained results, as a tradeoff, tell that it covers works perfectly.

Trollies show more biofilm patterns than surgical tables in spite of they follow the same sterilizing protocol. The reason behind that is the frequency of cleaning as well as sterilizing doses and materials are different which affect biofilm growth rate.

Shelves and drawers, ventilator machines, and liquid suction machines admit approximately the same biofilm levels. The biofilm on those surgical room parts sourced according to the same process. That is, primary cleaning with towels and water with detergents followed by ordinary disinfection protocol. In every sterilizing cycle, free microorganisms in free form killed while those in biofilm resist.

Photometry results gave further confirmation of biofilm quantification. This technique could be summarized by putting biofilm coupons between a light source and light intensity measuring device. The more the biofilm form the less the light penetrate the coupon. It provides cheapness, abundance, and representativity to the measurement technique. The last version of such method was introduced recently [19] and used in this work to confirm what would be found by the viable count method. Also, Figure 2 emphasizes what we got in figure 1. It gave a horizontal mirror image of it as the principle of measurement for the two figures are inversely proportional.

In addition to photometry, microscopy gives information about the development of biofilm under the conditions that relate to current disinfection protocols. The development is slow as the whole process lacks the optimum conditions (an aqueous environment, higher nutrients etc.) for rapid biofilm formation [31, 32].

From these results, we conclude that the process of biofilm formation in the areas studied takes the following steps: primary cleaning spreads infected water on surfaces. Microorganisms start to form biofilms as a result of the first stress, drought. After this, when disinfection is applied, a second stress drives bacteria to further protect themselves, thus strengthening biofilm building. In subsequent cleaning, in the second day, for instance, more bacteria are introduced, which give the biofilms new microbial diversity and an unstressed environment. The same cycle starts again. This, if compared with ordinary, e.g. in-pipe, biofilm formation, explains why it is slow. The biofilms build intermittently rather than continuously.

From a practical point of view, this phenomenon is absent from the minds of sterilization officers in the health sector. Even though such microbial aggregates were first described once the microscope was invented in the 17th century, the term biofilm was not coined and introduced until 1978 [33]. At that time, the sterilization techniques of health institutions concentrated on the well-known procedures that targeted planktonic bacteria, not the biofilm ones. The other factor that reinforces such techniques is the negative results that can be obtained with traditional swab tests. When they are exposed to biocides, bacteria go deep into biofilm to protect themselves. In this stage, swabs would not find any bacteria. While it is logical to get such outcomes when the behavior of bacteria in biofilms is understood, the hidden risk needs to be pointed out.

After depletion of bacteria due to biocide application, microorganisms return to the surface and spread again. Because the bacteria are going through the several stages (attachment, irreversible attachment matrix formation and maturation and detachment) at different times on different surfaces, some surfaces in operating rooms reveal bacterial pollution in some tests and others do not. The problem is not poor disinfection procedures; it is ignoring dealing with biofilms as a new disinfection problem.

Even the previous studies were with a limited number, they rung the alarm about such a problem [34-37]. During the last two decades this negative phenomenon attracted the scientist's attention and care. All of them, reached the conclusion of the existence of biofilm on various surfaces in operating rooms. In Iraq we suffer from lack of information about this issue. This work addresses the extend of the engagement of biofilm in surfaces in infections of post-surgery. The obtained results confirmed the research hypothesis as well as

confirming the results of previous works with some deviation in results due to many factors such as workers awareness and used cleaning materials and strategy.

5. Conclusions

Biofilms pose a risk to hospitals, especially in surgical operating rooms, labs, and clinics. This is often a direct result of following sterilization protocols designed to target planktonic bacteria, not biofilm. Bacterial biofilms are more resistant to antimicrobial therapies than microorganisms that float freely. Unfortunately, biofilms, once established, tend to increase their resistance. To provide that once they are treated with biocide, they will be more productive.

The recommendation in the face of such problems is to develop a protocol specifically to fight biofilm formation in health and medical facilities. Such a protocol to be used before or accompanying any traditional disinfection strategy.

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