Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [10]:1-23
[http://scholarsresearchlibrary.com/archive.html]

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation of Silver Nanoparticles as Disinfectant in Surgical Operating Rooms

Mahmoud M Bakr[1], Samar M Solyman[2], Ahmed I. El-Batal[3], Amro M Hanora[2*]

[1]*Suez Canal University Educational Hospital, Ismailia, Egypt*

[2]*Department of Microbiology & Immunology, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt*

[3]*National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt*

****Corresponding author:** Amro M Hanora, Department of Microbiology & Immunology, College of Pharmacy, Suez Canal University, Ismailia, Egypt. E-mail: a.hanora@pharm.suez.edu.eg*

ABSTRACT

Objectives: *This study aims to detect the presence of bacterial biofilm in the surgical operating rooms in Suez Canal University Hospital, and evaluate the activity of silver nanoparticles (AgNPs) as an antibiofilm agent and its effect in bacterial colony count in surgical operating rooms.*

Methods: *Frequently touched surfaces in five surgical operating rooms were, swabbed with dry sterile cotton swabs such as air supply vents, beds, anesthesia devices' surfaces, doors, and walls. The bacterial samples were tested for biofilm production by microtitre plate method. Then the biofilm producers were identified using VITEK® 2 system. Silver nanoparticles were tested to inhibit the biofilm formation and to eradicate pre-formed biofilm in microtitre plate and the effect was measured spectrophotometrically. After that silver nanoparticles were tested to reduce the aerobic colony count on frequently touched surfaces besides walls and floors in operating rooms by determination the colony count before and after use of silver nanoparticles.*

Results: *A total of 71 samples obtained from five surgical operating rooms, 19 samples were found to be strong biofilm producers and 25 were moderated biofilm producers. Silver nanoparticles and sodium hypochlorite showed no statistical differences in in vitro tests of antimicrobial activity and antibiofilm activity. Silver nanoparticles (AgNPs), in concentration 150 ppm, showed significant decrease in aerobic colony count on operating rooms frequently touched surfaces after use of AgNPs, and significant reduction of aerobic colony count (ACC) on walls and floors in comparison with sodium hypochlorite.*

Conclusion: *Silver nanoparticles are more efficient in reduction of ACC in comparison with sodium hypochlorite.*

Key words: Silver nanoparticles, hypochlorite, biofilm, Surgical Operating Rooms

INTRODUCTION

Infection control policy is a set of practices and measures applied to all patient care units in healthcare setting to provide high-quality health care for patients and a safe working environment for those who work in healthcare settings **[1]** Cleaning and disinfection of surfaces in health care setting is important to prevent exposure to environmental or airborne pathogens may result in infections with significant morbidity and mortality and to bring outbreaks under control **[2].**

A biofilm is a complex multicellular community formed at surfaces and interfaces **[3]**. It occurs in nearly every moist environment where sufficient nutrient flow is available and surfaces attachment can be achieved [4]. Biofilms have been found to protect the microbial community from environmental stresses. Therefore, the formation of biofilm allows the bacteria to develop resistances to bacteriophages, amoebae, chemically diverse biocides, host immune responses, and antibiotics through the production of a protective cover, the extracellular polysaccharide substances. Bacterial biofilm particularly problematic due to its high antimicrobial resistance tendency causing numerous chronic infections such as chronic osteomyelitis, and chronic pneumonia in patients with cystic fibrosis. In addition, infections associated with the use of biomaterial such as; intravascular catheters, urethral catheters, and prosthetic heart valves **[5].**

Nanotechnology is a new strategy in fighting bacteria due to unique properties of metal in form of nanoparticles. The high surface-to-volume ratio of nanoscale materials is associated with several novel and desirable properties compared with the corresponding bulk materials. These properties include chemical, mechanical, electrical and optical characteristics such as light absorption and conductivity, as well as catalytic and biological activity **[6,7]**. Silver nanoparticles (AgNPs), especially, have a strong antibacterial effect against Gram-negative bacteria, Gram-positive bacteria, fungi, and viruses [8-12]. They are commonly used in water filters for inhibition of biofilm or additional level of treatment, also used in hospital water systems as a secondary disinfectant to reduce the level of chlorine in drinking water supplies **[26]** Although, the inherent resistance of biofilm against antimicrobial and host immune system, it was observed that silver nanoparticles in different particles size and concentration have significant log reduction and inhibition of biofilm [13,14,15]

This study aimed to detect the bacteria that contaminating frequently touched surfaces in the surgical operating rooms in Suez Canal University Hospital and determination their ability to produce the biofilm, then demonstrate the antibacterial and antibiofilm activity of silver nanoparticles on the isolated strains and evaluate its use as disinfectant appropriate for bacterial colony count reduction in surgical operating rooms.

Materials and methods

Table: list of materials and media

Media/ chemical name	Source
Nutrient agar	*lab m LTD, UK*
Nutrient broth	*lab m LTD, UK*
Trypticase soy agar	*lab m LTD, UK*
Trypticase soy broth	Oxoid LTD, UK
40 % Glycerol	*Loba chemia, India*
0.1% crystal violet solution	Sd Fine-chem LTD, India
Muller-Hinton broth	*Oxoid limited, UK*

Methods

Collection of samples

There are 7 surgical operating rooms in Suez Canal University hospital for various kinds of surgeries. The room number 7 was specialized for ophthalmic surgeries. Each room is about 30 m^2 in space and 2.5 m Hight, and containing the surgical bed in the middle of the room, and anesthesia device. In one side of the wall, there are shelves impeded in the wall used to store I.V. infusion solutions and any other devices and tools. In the roof, there are 1 or 2 hanged torches and 4-6 air supply vents.

The samples were collected by swabbing frequently-touched surfaces in surgical operating operation rooms in Suez Canal University by using dry sterile cotton tipped swabs **[16]**, after 24 hours of usual cleaning procedures with 0.5% sodium hypochlorite. The swabbing was done once a week from October 2014 to November 2014. Surfaces swabbed, area of swabbing, swabbing time, and number of swabs from surfaces is illustrated in (Table 2).

Table 1: Description of sampling

Swabbed surfaces	Area of swabbing	Swabbing time	Number of swabs	Location of sampling
Surgical bed	20 cm* 20 cm	30 seconds	2	From top and bottom of the bed
Anesthesia device	10 cm* 10 cm	30 seconds	2	Screen surface and button
Shelves	20 cm* 20 cm	30 seconds	2	From two sites of the shelves
Floor	20 cm* 20 cm	30 seconds	2	Beside the bed and from corners
Walls	20 cm* 20 cm	30 seconds	2	From the middle of the wall
Door	20 cm* 20 cm	30 seconds	2	from each side of the door

Isolation and purification of samples

The swabs were transferred to the lab within 30 minutes of sampling. Each swab immersed in 5 ml of saline, then mixed well, and then swabs 0.5 ml were inoculated into a nutrient agar (NA) and trypticase soy agar (TSA) plates, then incubated at 37^0c for 24 hours. Purification of isolated colonies by transferring mixed plates on new plates until get pure colonies. The isolated colonies were saved in 40% glycerol nutrient broth and kept at -20 ^{o}c [17]. The isolated purified samples coded with room number and swabbed site.

Detection of biofilm production using microtitre plate method (STEPANOVIC, et al. 2007)

An overnight bacterial growth on nutrient agar plates was inoculated into trypticase soy broth supplemented with 1% glucose, then incubated for 24 hours at 37^{o}C. The culture was diluted 1:100 with fresh medium to make the bacterial suspension. One hundred microliters of bacterial suspensions were added to 96-well microtitre plate, and incubated for 24 hours at 37^{o}C. After incubation, the contents of the plate were discarded, then plates washed four times with 200 µl normal saline to remove planktonic cells. Biofilm formed by bacteria adherent to the inner wall of the wells fixed by 200 µl of 2% sodium acetate for 5 minutes, then washed with distilled water. Fixed biofilm was stained by 200 µl of 0.1% crystal violet aqueous solution for 10 minutes. Excess stain removed by washing with distilled water, and left to dry. After drying, 100 µl of distilled water and 100 µl of 1N HCl were added as coloring agent. The optical density of the stained biofilm was obtained by ELISA autoreader at wavelength 450 nm – 630 nm. The test was done in duplicate, obtaining the average of absorbance and the interpretation of biofilm production was done according to Stepanovic criteria Table 2. Positive control used was *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis,* which obtained from faculty of pharmacy, Suez Canal University Ismailia, Egypt.

Table 2: Stepanovic Criteria

non/ weak producer	OD ≤ ODc
	ODc ≤ OD ≤ 2*ODc
Moderate producer	2*ODc ≤ OD ≤ 4*ODc
strong producer	OD ≥ 4*ODc
ODc (optical density cut-off of negative control) = average of OD of negative control + 3* Standard deviation of negative control	

Identification of strong and moderate biofilm producer strains

Biofilm-producing isolates identified as Gram-positive or Gram-negative using Gram stain set. Identification of Biofilm-producing isolates was done using VITEK® 2 Systems Version 07.01 (BioMérieux), and the tests were run using VITEK® 2 ID cards.

Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) and Polyvinylpyrrolidone (PVP) solutions were used for the synthesis of AgNPs. The modified method of (El-Batal, et al. 2016 a and 2016 b) was applied as follows: 10 ml of 0.5% PVP solution was mixed with 90 ml of 5.0 mM $AgNO_3$ solution and. The mixtures were exposed to gamma radiation at dose 25 kGy at room temperature. After irradiation,

the produced AgNPs were characterized according to (El-Batal, et al., 2016a and 2016b). The method of irradiation was conducted at National Center for Radiation Research and Technology (NCRRT). The facility practiced was 60 Co-Gamma chamber 4000-A-India. Irradiation was administered at a dose rate 2.9 kGy/ hour at the time of the experiment.

***Determination of minimum inhibitory concentration (MIC) by microdilution method on 96-well microtitre plate* [18]**

In a sterile 96-wells microtitre plate, 100 µl of silver nanoparticle solutions in concentration 500 ppm added into the first row of the plate and 50 µl of Mueller-Hinton broth were added into the rest rows. Two-fold serial dilutions of silver nanoparticle solutions were made by drawing up 50 of silver nanoparticle solution from the first row into the next one and then mix well, after that draw up 50 µl of the mix and added to the next well and so on. The concentrations of silver nanoparticles in the wells from row (A) to the row (F) of the plate was as follows 500, 250, 125, 62.5, 31.25, and 15.625 ppm. The rows (G) and (H) was used as negative and positive controls, respectively. The positive control was inoculated with strains *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis*, which obtained from faculty of pharmacy, Suez Canal University Ismailia, Egypt, while the negative control wells were Mueller-Hinton broth and disinfectant solution with no bacterial suspensions. Preparation of bacterial suspension made from an overnight growth of bacterial isolates was as follow; two separated colonies were collected and suspended in saline matching 0.5 McFarland turbidity standard. Aliquot (50 µl) of each bacterial suspension were inoculated into wells of microtitre plate to obtain a final volume of 100 µl in each well of the plate, then incubated for 24 hours at 37^{o}C. The MIC of silver nanoparticles detected as the lowest concentration cause inhibition of bacterial growth and that indicated by no turbidity was observed in the wells.

Testing of antibiofilm activity of silver nanoparticles

Testing the activity of inhibition of biofilm formation [18]

This method depends on visual observation of biofilm formed in the wells of microtitre plate in presence of silver nanoparticles solutions by staining with 0.1% crystal violet solution as indication of bacterial growth. The antibiofilm activity of silver nanoparticles in concentrations 500 ppm, 250 ppm and 125 ppm was tested in comparison with sodium hypochlorite solution in concentration 5000 ppm (0.5%), as a standard disinfectant in the policies of infection control in healthcare facilities in Suez Canal University hospital. Three microtitre plates, each containing 100 µl of silver nanoparticle solutions in concentrations 500 ppm, 250 ppm, and 125 ppm, respectively. Fifty microliters of Mueller-Hinton broth were added into the wells of plates. Aliquot (50 µl) of bacterial suspension of 44 strains of strong and moderate biofilm producers were inoculated into wells of a microtitre plate and incubated at 37^{o}C for 24 hours. The inhibition effect indicated by detecting the biofilm formation using a crystal violet stain. After 24 hours of incubation, contents of the wells were discarded, and the wells were washed three times with 200 µl normal saline. Adherent biofilms had been fixed by 200 µl of 2% sodium acetate for 5 minutes, and washed with distilled water. Biofilms were stained by 200 µl of 0.1% crystal violet aqueous solution for 10 minutes, and excess stain was removed by washing with distilled water, then add 200 µl of distilled water to dissolve the stain. colorless solution in the well indicating no biofilm formed, while appearance of blue color of crystal violet stain indicates biofilm growth. This test run in comparison with negative and positive control. The standard strains used as a positive control were *Staphylococcus aureus, Escherichia coli, and Bacillus subtilis*, which obtained from department of microbiology and immunology, Suez Canal University.

Chi square test was used to find the difference in inhibition of biofilm formation between silver nanoparticles in concentrations 500 ppm, 250 ppm, and 125 ppm and between silver nanoparticles and sodium hypochlorite solutions in concentration 5000 ppm (0.5%).

Testing the activity of silver nanoparticles to eradicate the formed biofilm [19]

This method is based on comparing the biofilm mass before and after the treatment with the antimicrobial agent and that indicating the ability of antimicrobial agent to eradicate the formed biofilm. The test had been performed by preparing 96-wells microtitre plates containing 100 µl of biofilm growing medium. Bacterial suspension made from an overnight growth of strong and moderate biofilm producer, then matching with 0.5 McFarland turbidity standard. 100 µl of bacterial suspension let to grow as a biofilm in microtitre plate for 24 hours at 37 ^{o}C. After incubation, content of the wells was discarded and plates were washed by 200 µl normal saline three times. After that, 100 µl of fresh medium and 100 µl of antimicrobial agents, silver nanoparticles 500, 250, and 150 ppm and sodium hypochlorite 5000 ppm, were added into the wells and incubated for another 24 hours at 37^{o}C. After incubation, content of the wells was discarded, and the wells were washed three times with 200 µl normal saline. The adhered biofilm fixed by 200 µl of 2% sodium acetate for 5 minutes, then washed with distilled water. After washing, the adherent biofilms were stained by 200 µl of 0.1% crystal violet aqueous solution for 10 minutes, and excess stain was removed by washing with distilled water. After that, 100 µl distilled water and 100 µl 1N HCl was added and each plate was duplicated. The stained biofilms were measured by ELISA reader at wavelength range 492 nm- 630 nm. Determine the percentage of inhibition by the following formula **[20]**.

$$\text{Percentage of inhibition} = \frac{OD\ of\ pretreatment\ control - OD\ of\ post\ treatment}{OD\ of\ pretreatment\ control} \times 100$$

Determination the reduction of Aerobic Colony Count (ACC) using silver nanoparticles 150 ppm and NaOCl 0.5% in surgical operating room

This test done through randomized control trial in the comparing of two procedures for cleaning the operating room. Using of silver nanoparticles on the frequently-touched surfaces (e.g. bed, door, anesthesia device surfaces) and non-critical surfaces (floors and walls) in concentration 150 ppm after cleaning with water only. The usual cleaning procedure by environmental service workers with sodium hypochlorite on non-critical surfaces. While there was no monitoring on the concentration of prepared solution or the cleaning procedure performed.

Five rooms of surgical operating rooms had been used to test the ability of AgNPs and sodium hypochlorite to reduce the aerobic colony count. Each room had been washed twice with each antimicrobial agent. Any other antimicrobial agent should not be used before washing. To avoid the physical and chemical factors that percent in the environment such as pH, temperature, relative humidity, and water hardness concentration of silver nanoparticles used increased from 125 ppm to 150 ppm.

The method was modified from the source [21]. The original method used several types of swabs and diluents for the detection of specific types of microorganisms, such as for detection of *salmonella* species buffered peptone water and selective enrichment medium should be used and for just enumeration the peptone saline used as diluent. In the current study used normal saline as diluent and plate count agar [22] as the growth medium.

The cleaning procedure using silver nanoparticles 150 ppm was as follow; at the end of working day, the operating room was cleaned using only water and remove the waste. Then the silver nanoparticles 150 ppm sprayed over the frequently touched surfaces mentioned in **Table 4** using pressure sprayer, beside floors and walls. The sprayer was moved back and forth to ensure covering the surface under test. The same steps done when cleaning with sodium hypochlorite, but the sodium hypochlorite was not used on frequently touched surfaces to avoid its damaging. Each room underwent assessment of aerobic colony count on frequently touched surfaces at the end of surgical working day and after 24 hours by swabbing. The experiment was repeated twice in each room.

The surfaces' swabs collected as the decontamination plan illustrated in and **Table 4**. After swabbing, swabs were immersed in 5 ml of saline for bacterial extraction, and then two plates of plate count agar were inoculated with 0.5 ml of bacterial suspension for each. The plates coded and incubated at 37^{o}C for 48 hours and checked for growth every 24 hours. The count was calculated using the following formula:

$$\boldsymbol{total\ viable\ count} = \frac{\boldsymbol{C}}{\boldsymbol{V} * \boldsymbol{N1} * \boldsymbol{D}} * \boldsymbol{N2}$$

Where: **C** is the sum of colonies on all plates counted; **V** is the volume applied to each plate in ml; **N1** is the number of plates counted at the first dilution; **N2** is the original volume of suspension in ml; And **D** is the dilution from which the first count was obtained e.g.10^{-2} is 0.01. If the swab is from a measured area the count can be divided by the area swabbed in cm^2.

Table 3: Room(s) decontamination plan

Date of ...			Room(s) Decontaminated with **sodium hypochlorite**	Room(s) Decontaminated with **silver nanoparticles**
Pretreatment swabbing	intervention	Post-treatment swabbing		
8/4/2016 Thursday	8/4/2016 Thursday	9/4/2016 Friday	-	3, 4
10/4/2016 Saturday	10/4/2016 Saturday	11/4/2016 Sunday	3, 4	-
15/4/2016 Thursday	15/4/2016 Thursday	16/4/2016 Friday	-	2, 7
17/4/2016 Saturday	17/4/2016 Saturday	18/4/2016 Sunday	2, 7	-
22/4/2016 Thursday	22/4/2016 Thursday	23/4/2016 Friday	-	3, 4, 5
24/4/2016 Saturday	24/4/2016 Saturday	25/4/2016 Sunday	3, 4, 5	-
29/4/2016 Thursday	29/4/2016 Thursday	30/4/2016 Friday	-	2, 7, 5
1/5/2016 Saturday	1/5/2016 Saturday	2/5/2016 Sunday	2, 7, 5	-

Table 4: Description of sampling before and after disinfectants interventions

Swabbed surfaces	Area of swabbing	Swabbing time (seconds)	No. of swab(s) from the surface from each room
Anesthesia device	10 cm* 10 cm	30	2
Surgical bed	20 cm* 20 cm	30	1
Door	20 cm* 20 cm	30	2
lamp	10 cm* 10 cm	30	1 (from room number 3 only)
Shelves	20 cm* 20 cm	30	2
Chair	10 cm* 10 cm	30	1(from room number 7 only)
Trolley	20 cm* 20 cm	30	1(from room number 7 only)
Floor	20 cm* 20 cm	30	2
Walls	20 cm* 20 cm	30	2

Statistical analysis

Chi-square test had used to evaluate difference in antimicrobial activity between the different disinfectants and reduction of aerobic colony count. The one-way ANOVA test was used to evaluate the difference in reduction of biofilm mass at $p < 0.05$. The analysis made using the SPSS program version 22.

RESULTS

Samples collection

One hundred and twelve swab samples were collected from five surgical operating rooms in Suez Canal University hospital. Sites of isolation and number of isolates as shown in (Table 5). Of which, 71 samples (63.39%) obtained as positive growth. The isolated samples were saved in 40% glycerol nutrient broth for further tests [23-29]**.**

Table 5: Isolated samples from swabbed surfaces

Swabbed Surface	Number of isolates
Anesthesia device	14
Surgical bed	19
Shelves	16
lamp	7
Door	7
Floors	3
Walls	5

Detection of biofilm production using microtitre plate

The biofilm production of the 71 isolates was as follow; Non- or weak biofilm producers were 27 isolates (38.02 %), 25 isolates (35.21 %) were moderate biofilm producers and 19 isolates (26.76 %) were strong biofilm producer. The positive control; *Staphylococcus aureus, Escherichia coli, and Bacillus subtilis*, showed moderate biofilm production. Figure 1 showed the density of the stained biofilm on microtiter plate.

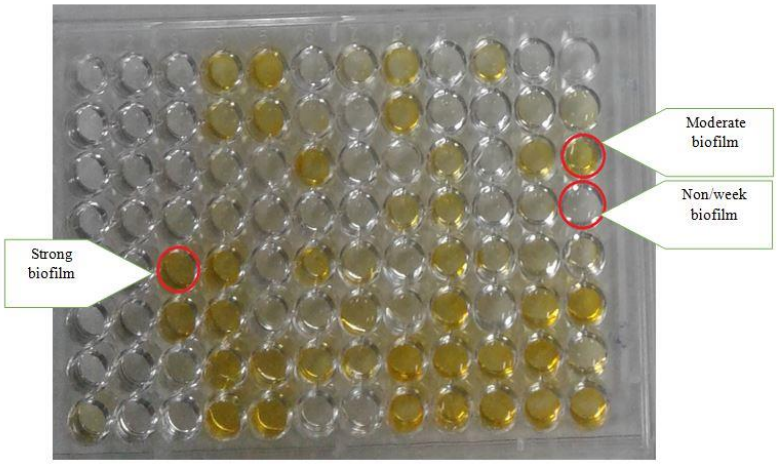

Figure 1: Testing of biofilm production on 96-wells microtitre plate. The yellow color appeared after addition of coloring agent to stained biofilm. The density of the color indicates the extent of biofilm production.

Identification of biofilm producing isolates

Identification of 19 strong-biofilm producers and 25 moderate biofilm producers by VITEK® 2 system with identification probability 86%-99%. The identified organisms illustrated in Table 6.

Table 6: Identified microorganisms

Identified microorganism	**Frequency**
Strong biofilm producers	
Staphylococcus aureus	2
Staphylococcus vitulinus	4
Staphylococcus lentus	6
Staphylococcus sciuri	3
Enterococcus columbae	1
Aerococcus viridans	1
Unidentified sample	2
Moderate biofilm producers	
Pseudomonas oryzihabitans	2

Staphylococcus sciuri	2
Staphylococcus lentus	9
Staphylococcus carnosus ssp carnosus	2
Aerococcus viridans	2
Unidentified samples	8

Synthesis of silver nanoparticle

Silver nanoparticles synthesis noticed by deep- yellowish brown color formation. Result of silver nanoparticles synthesis at 25.0 kGy gamma irradiation dose showed maximum absorption (3.123) at the wavelength (433nm) [30-33].

Determination of MIC

The MIC was determined by absence of turbidity in the well of the lowest concentration, that indicating the growth inhibition. So as shown in Figure 2, silver nanoparticles in concentrations 500 ppm and 250 ppm showed no turbidity in 71 of isolates, while silver nanoparticles in concentration 125 ppm showed no turbidity in 64 isolates. From the previous, the MIC obtained as 125 ppm [34-37].

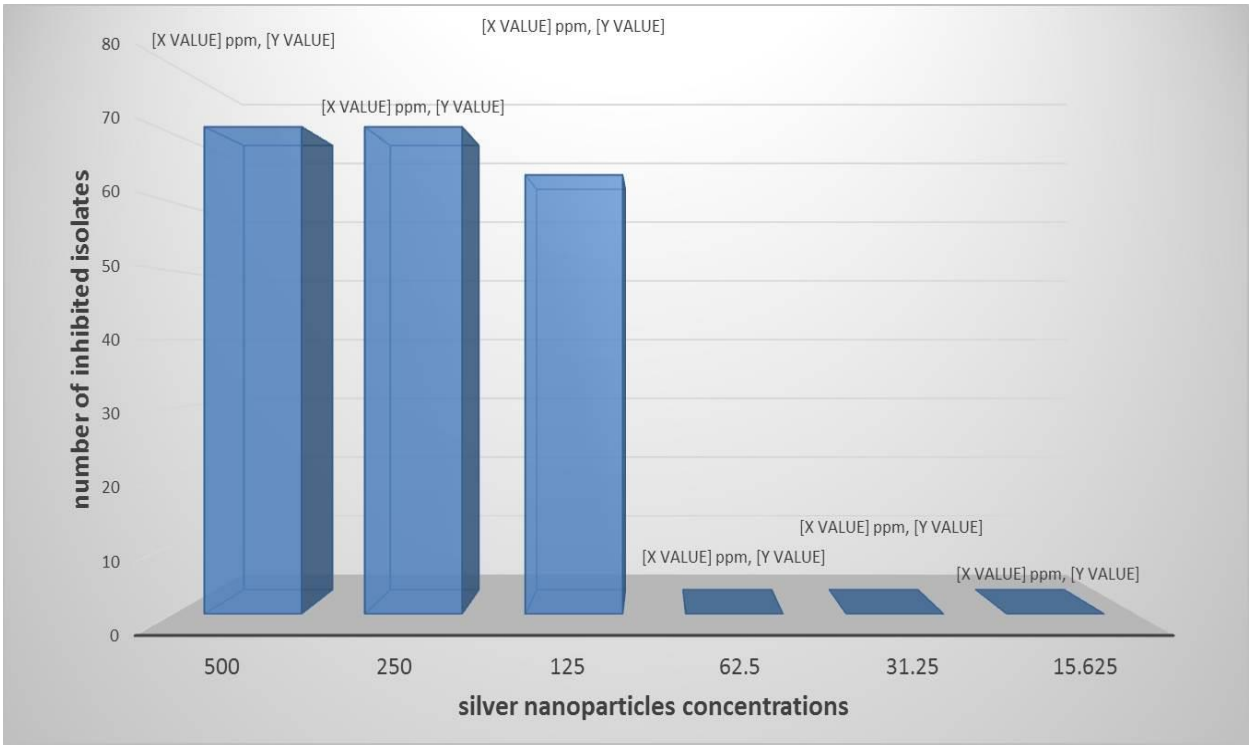

Figure 2: MIC plotting chart.

Testing of antibiofilm activity

Testing the activity of inhibition of biofilm formation

This test is measuring the ability of antimicrobial agents to prevent the formation of biofilm. The results of the test as shown in Table 7 [38-42].

Table 7: Inhibition of biofilm formation by antimicrobial agents

Antimicrobial agent		Inhibited isolates
Silver nanoparticles	500 ppm	44
	250 ppm	44
	125 ppm	42
Sodium hypochlorite	5000 ppm (0.5%)	40

The Chi square analytical test showed that the difference in inhibition of biofilm formation between silver nanoparticles in concentrations 500 ppm, 250 ppm, and 125 ppm was not significant (p-value= 0.440771), and between silver nanoparticles and sodium hypochlorite (5000 ppm) was also not significant (p-value= 0.233763) at $p < 0.05$.

Testing the activity of eradication of preformed biofilm

When silver nanoparticles used to eradicate the formed biofilms of 44 isolates, concentration 500 ppm showed inhibition by 68%, concentration 250 ppm showed inhibition by 56.81%, and concentration 125 ppm showed inhibition by 59.09%. While sodium hypochlorite in concentration 5000 ppm, it showed inhibition of the 44 isolates by 45%. Figure 3 showed the average of percentage of inhibition of biofilm masses by the different disinfectants.

Statistical test performed using One-way ANOVA test. The difference in reduction of biofilm mass between silver nanoparticles in concentrations 500 ppm, 250 ppm, and 150 ppm (*P*-value 0.809) and between silver nanoparticles and sodium hypochlorite in concentration 5000 ppm (*P*-value 0.928) were not significant at $p < 0.05$ [43-46].

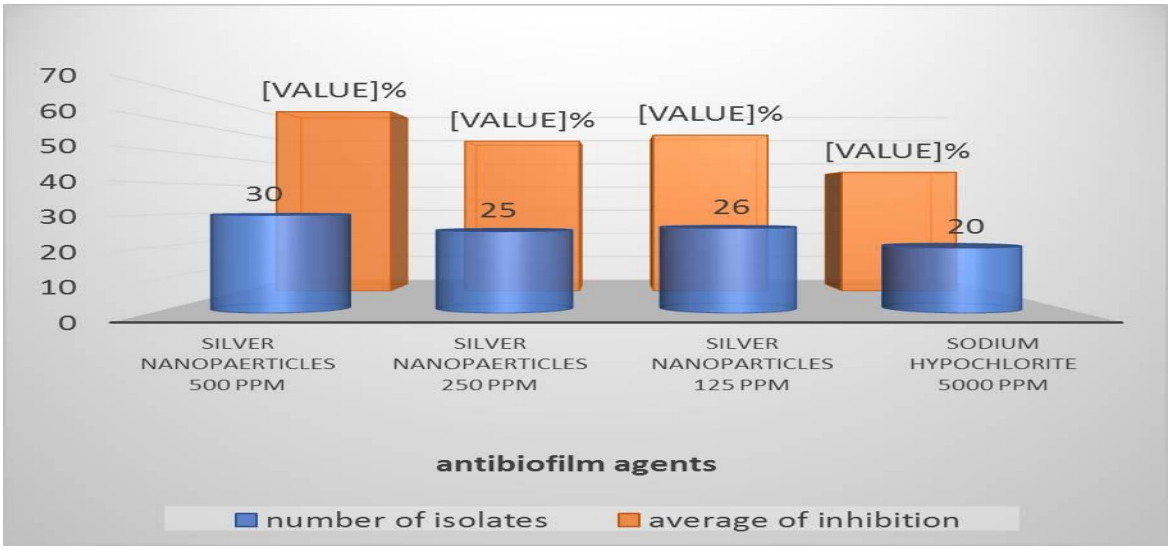

Figure 3: Number of eradicated biofilms and percentage of eradication.

Determination the reduction of aerobic colony counts (ACC) using silver nanoparticles 150 ppm and NaOCl 0.5% in surgical operating room

Silver nanoparticles in concentration 150 ppm showed decrease in aerobic colony count on frequently touched surfaces by 95.6 % against increase in aerobic colony count by 14.09 % on same surfaces when no intervention taken for decontamination, as sodium hypochlorite should not be used on these surfaces to avoid its damage. the reduction of aerobic colony count of silver nanoparticles 150 ppm was statistically significant (*P*-value= 0.00001, p< 0.05), Figure 4 shows aerobic colony count represented by CFU/ cm^2 on frequently touched surfaces in the operating rooms before and after disinfection with silver nanoparticles 150 ppm and the colony count with no intervention.

When silver nanoparticles used on floors and walls showed decrease in aerobic colony count by 85 % against increase of aerobic colony count when decontaminated by sodium hypochlorite by 252.5 %. Figure 5 shows the aerobic colony count on floors and walls before and after decontamination with silver nanoparticles 150 ppm and sodium hypochlorite 5000 ppm. The microorganisms that contaminated the frequently touched surfaces are illustrated in

Table **8** [47-52].

Table 8: Microorganisms that contaminating the frequently touched surfaces in surgical operating rooms

Surface swabbed	Strong biofilm producers	Moderate biofilm producers
Anesthesia device	*Staphylococcus aureus* *Staphylococcus sciuri* *Aerococcus viridance*	*Staphylococcus lentus*

bed	*Staphylococcus vitulinus* *Staphylococcus lentus*	*Staphylococcus sciuri* *Staphylococcus carnosus spp carnosus*
door	*Enterococcus columbae* *Staphylococcus vitulinus*	-----
shelves	*Staphylococcus lentus*	*Pseudomonas oryzihabitans* *Aerococcus viridans*

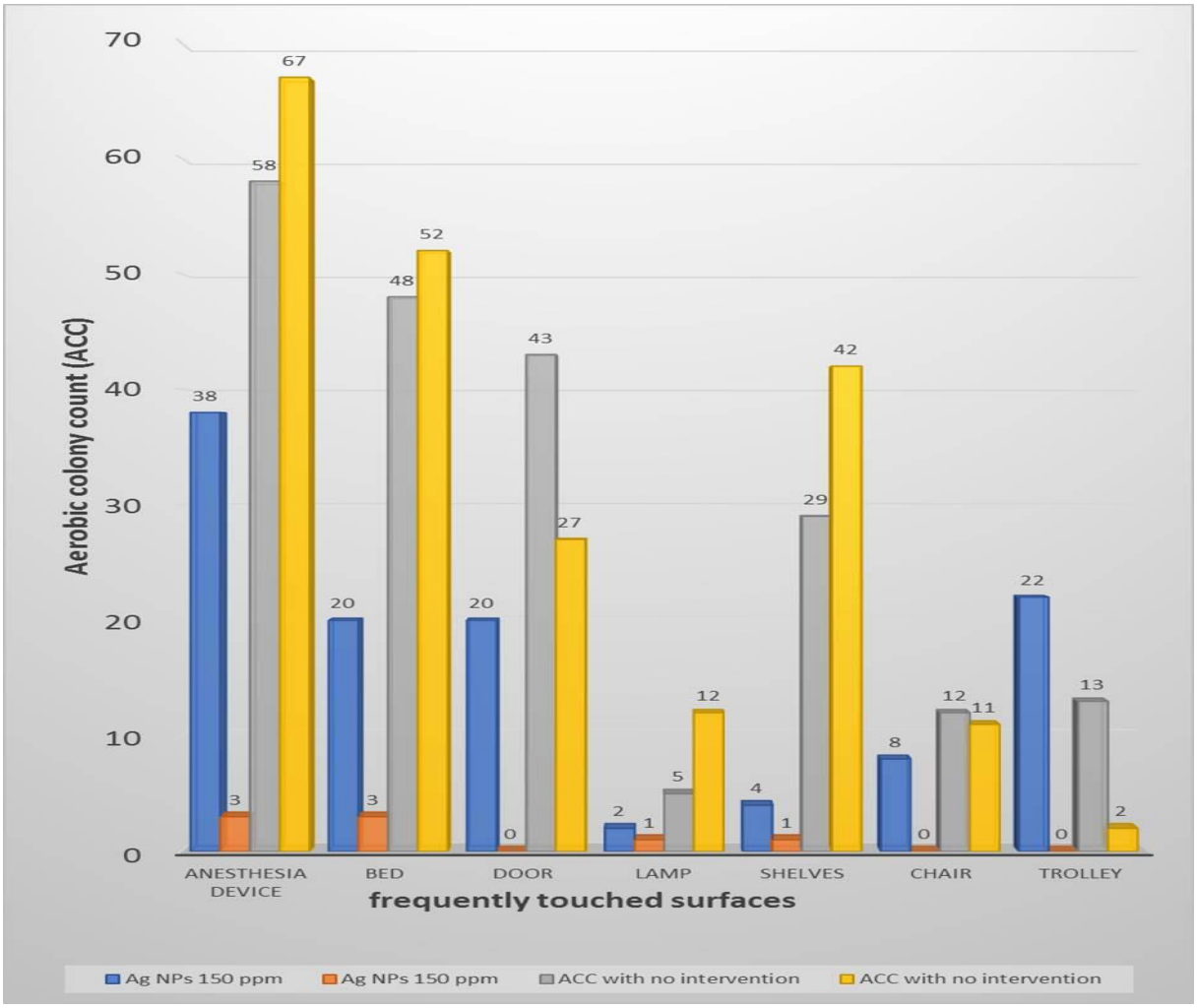

Figure 4: The effect of silver nanoparticles 150 ppm on ACC on frequently touched surfaces against no intervention taken on the same surfaces.

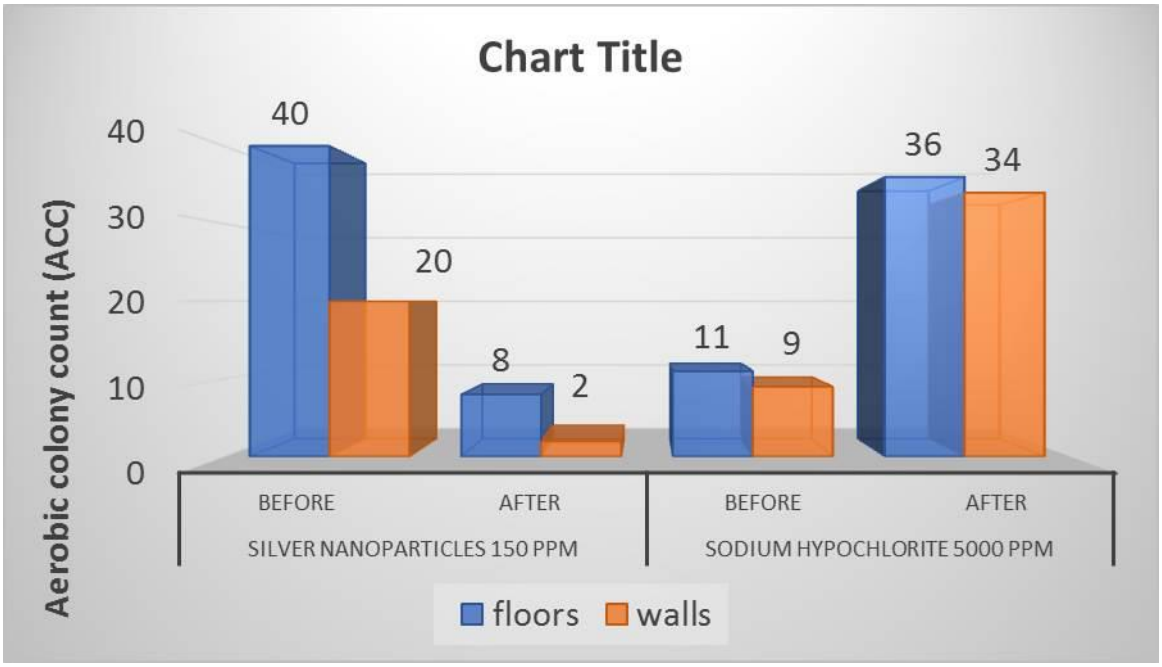

Figure 5: Reduction of ACC on floors and walls by silver nanoparticles 150 ppm and sodium hypochlorite 5000 ppm (0.5%).

Discussion

This study aims to detect the presence of biofilm-producing bacteria in the surgical operating rooms in Suez Canal University hospital, and evaluate the cleaning protocol of surgical operating rooms by using environmental culture method. In this research, silver nanoparticles generated to use as disinfectant for surfaces decontamination in surgical operating rooms. There are no studies previously provided a use of the silver nanoparticles for the disinfection processes of surgical operating rooms.

In the present study, Samples collected by swabbing the frequently touched surfaces of surgical operating rooms using dry sterile cotton swabs with a sensitivity 63.39 %. This data was agreeing with results of **[53]** and **[54]** who found that the dry sterile cotton swaps have a sensitivity about 60 %- 62 % of extraction of microorganisms from contaminated surfaces. This method in considered simple and does not need for special techniques, so used for qualitative detection of bacteria that contaminating surfaces.

This study used microtitre plate method for quantitative evaluation of biofilm formation, as this method considered standard in evaluation of biofilm activity. The results obtained from this method was 61.97 % were biofilm producers and that resembled that by **[55]** who tested 152 clinical isolates of *Staphylococcus* spp. to produce biofilm by Congo red agar (CRA), microtitre plate method and tube method (TM) methods. The results showed that 97.1% of strains assessed as biofilm producers

with the use of microtitre plate method, 73.6% by TM, and only 6.8% in the case of CRA method, thus the authors concluded that the most sensitive method for biofilm formation detection was the microtitre plate method. It is also as the results of **[90]** who found that only 7.4% of analyzed coagulase negative staphylococci strains yielded positive reactions in the CRA method. While by microtitre plate method showed that 20.5% of isolates could produce biofilm. These studies were strongly suggested that the use of microtitre plate method for the evaluation of biofilm formation ability by strains isolated from hospital environment [56-58].

The antimicrobial activity of silver nanoparticles on environmental isolates showed no difference than that of sodium hypochlorite. These results resampled with study of **[59].** Who tested bactericidal effect of silver nanoparticles as a final irrigation agent in endodontics. Silver nanoparticles in concentration 537 µg/ ml (537 ppm) compared with sodium hypochlorite 2.25 % (25,000 ppm) in prevention and treatment of endodontic infections. The results showed that nanoparticles of 10 nm and the sodium hypochlorite at 2.25% were effective for eliminating *E. faecalis*, with nonsignificant difference between them. The differences between current study and this study is, the microorganisms isolated from surface of uniradicular extracted dental organs intentionally contaminated with *Enterococcus faecalis* standard strain, while the current study tested the effect on microorganism isolated from environmental surfaces from operating rooms [60-66].

Lack of standardization makes a comparison of the silver nanoparticles tests results difficult, and can lead to the generation of conflicting data between studies since the experimental outcome is strongly dependent on the assay conditions and materials employed in the testing. Crystal violet was widely used for staining methods applied to biofilm quantification as it is inexpensive and measures the effects on total biomass of biofilms **[67]**. The antibiofilm activity of silver nanoparticles tested to be used for surfaces that might be able to aid the growth of biofilm by preserving moisture or porous surface [68-70].

The use of silver nanoparticles in higher concentration (150 ppm) rather than the MIC concentration (125 ppm) in the test of reduction of aerobic colony count to avoid the physical and chemical factors that percent in the environment such as pH, temperature, relative humidity, and water hardness. These factors could affect the efficacy of silver nanoparticles solution and reduce its potency **[71]**.

The cleaning procedure of surgical operating rooms was using sodium hypochlorite according to Policies of infection control in healthcare facilities in Suez Canal University hospital. Sodium hypochlorite was used only on walls and floors and other surfaces cleaned only with wet cloths. In this study, the cleaning protocol evaluated by swapping these surfaces before and after cleaning, it found that increase in aerobic colony count by 14.09%. This result resembles with the In vitro study by **[40]** who

used sodium hypochlorite in intensive care unit. The results showed that even the exposure to hypochlorite at concentration 20,000 ppm, S. aureus cells were re-growing on prolonged incubation. The contamination of surfaces in surgical operating rooms of Suez Canal university hospitals by pathogenic microorganisms as shown in [72-76]

Table **8**, may be for many reasons; first, the cleaning protocol in the infection control policies and practices of Suez Canal university hospital [77] have some limitations in the cleaning procedure of blood spills, that is the blood spills removed first by absorbed cotton, then using sodium hypochlorite 5000 ppm for disinfect the surface. This procedure did not document the use of detergent before the disinfection. Second, the infection control policies and practices of Suez Canal University hospital did not document a procedure for cleaning of surfaces that could not be decontaminated by sodium hypochlorite such as beds and anesthesia devices, and that made the cleaning process varied as the cleaning worker. The increase that observed in aerobic colony count after cleaning with sodium hypochlorite may be due to low training and education on the updated guidelines of infection control for the environmental services workers, there were no monitoring on the disinfectants preparation and use, but just visual auditing [78-80].

In the current study, Silver nanoparticles was applied on semi-critical surfaces in operating rooms such as; anesthesia devices, beds, chairs and tables. The result was aerobic colony count inhbition on theses surfaces by 95.6%. sodim hypochlorite could not used on these surfaces to avoid its damage due to the corrosive effect on metal and destruction effect on fabrics. This result agree with that of **[81]** who tested Dettol® effect on viablity of microorgansims causing nosocomial infections. Different concnentrations of Dettol® had been used, and there was significant decrease in colony count. The difference between current study and previous study is that the tested effect of reduction of aerobic colony count in situ while the other study had used in vitro tests under controoled conditions. Besides, study had found that there is a relationship between multidrug resistance and disinfectant resistance, but there are no evidence on resistance against silver nanoparticles **[82]**. This result also resembles another study by **[83-86],** who used in vitro tests to evaluate a comercial silver-based disinfectant (AgION® silverclene™) on clinical isolates from hospitalizad patients. The results shoewd signficant decrese (78%) in growth. This current study differs from the other study by the using of *in situ* test against *in vitro* tests under controlled conditions.

Silver nanoparticles have a higher effect on reduction of aerobic colony count on any types of surfaces and have wide antimicrobial spectrum with low tendecy to develop resistance. In addition to its growing applications in healthcare facility and low or ignorent toxicity on human or hamaging effect on inanimate objects in comparison with sodium hypochlorite. So, it is obvieus that the silver nanoprtices is superior to be used in decontamination of surfaces in sterile rooms [86-94].

Conclusion

Silver nanoparticles-based disinfectant is superior to sodium hypochlorite due to its disadvantages of hypochlorite solution. Silver nanoparticles had found better antimicrobial and antibiofilm activity with higher stability than sodium hypochlorite. This study recommends the use of AgNPs as disinfectant in steril rooms.

References

1. Australian Commission for Safety and Quality in Health Care. AUSTRALIAN GUIDELINES FOR THE PREVENTION AND CONTROL OF INFECTION IN HEALTHCARE. **2010.**
2. Carroll, Karen C., "Pseudomonas, Acinetobacters, and Uncommon Gram-negative Bacteria." Chap. 16 in Jawetz, Melnick and Adelberg's Medical microbiology, by Geo F Brooks, Karen C Carroll, Janet S Butel, Stephen A Morse and Timothy A Mietzner, **2012.** 249.
3. Kolodkin-Gal, IIana., et al. "A self-produced trigger for biofilm disassembly that targets exopolysaccharide." **2012.** 684-692.
4. Singh, R., Debarati, Paul., and Rakesh, J., "Biofilm: implications and bioremediation." *Trends In Microbiology*, **2006.** 14(9): 389-397.
5. Maric, Svjetlana., and Jasmina, Vranes., "characteristics and significance of microbial biofilm formation." *periodicum biologorum*, **2007.** 115-121.
6. Rai, MK., et al. "Silver nanoparticle: the powerful nanoweapon against multidrug resistant bacteria." *journal of Applied Microbiology*, **2012.** 112: 841-852.
7. Schacht, VJ., et al. "Effect of silver nanoparticles on microbial growth dynamics." *Journal of Applied Microbiology*, **2012.** 114: 25-35.
8. Fewtrell, Lorna., Silver: water disinfection and toxicity. center for research into environment and health. **2014.**
9. Guzman, M., Jean, D., and Stephne, Get. "Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria." Nanomedicine, **2012.** 37-45.
10. Markowska, Katarzyna., Anna, NG., and Krystyna IW., "Silver nanoparticles as an alternative strategy against bacterial biofilms." *Journal of the Polish Biochemical Society*, **2013.** 60(4): 523-530.
11. Lander, TF., Armando, Hoet., and Thomas EW., "Swab Type, Moistening, and Preenrichment for Staphylococcus aureus on environmental surfaces." *Journal Of Clinical Microbiology*, **2010.** 2235–2236.
12. Bastos, MF.,. "Identification of new staphylococcins with potential application as food biopreservatives." *food control*, **2013.** 313-321.

13. Jiang, lin. "COMPARISON OF DISK DIFFUSION, AGAR DILUTION, AND BROTH MICRODILUTION FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING OF FIVE CHITOSANS." august: 33-34.
14. Kostenko, Victoria., et al. "Impact of silver-containing wound dressing on bacterial biofilm viability and susceptibility to antibiotics during prolonged treatment." *Antimicrobial agents and chemotherapy*, **2010.** 5120-5131.
15. Namasivaym, et al. "Biofilm inhibitory effect of silver nanoparticles coated catheter against Staphylococcus aureus and Evaluation of its synergistic effect with antibiotics." *International Jornal of Biological and Pharmaceutical Research*, **2012.** 3(2): 259-265.
16. Microbiology Services; Food, water, and Environmental Microbiology. Detection and enumeration of bacteria in swabs and other environemntal samples. London, England: Public Health. **2014.**
17. Moore, G., Christopher JG., and Louise, F., "A comparison of traditional and recently developed methods for monitoring surface hygiene within the food industry: A laboratory study." Dairy, *Food and Environmental Sanitation*, **2001.** 478-488.
18. Lander, TF., Armando, Hoet., and Thomas EW., "Swab Type, Moistening, and Preenrichment for Staphylococcus aureus on environmental surfaces." *Journal Of Clinical Microbiology*, **2010.** 2235–2236.
19. Thom, KA., et al. "Comparison of Swab and Sponge Methodologies for Identification of Acinetobacter baumannii from the hospital environment." *Journal of Clinical Microbiology* (American Sociaty for Microbiology), **2010.** 50(6): 2140-2141.
20. Mathur, T., et al. "detection of biofilm formation among the clinical isolates of staphylococci." *Indian Journal of Medical Microbiology*, **2006.** 25-29.
21. Wojtyczka, RD., et al. "Biofilm Formation and Antimicrobial Susceptibility of Staphylococcus epidermidis Strains from a Hospital Environment." International Journal of Environmental Research and Public Health 11: 4619-4633.
22. Gonzalez-Luna, Pedro-IV., et al. "Bactericide Effect of Silver Nanoparticles as a Final Irrigation Agent in Endodontics on Enterococcus faecalis: An Ex Vivo Study." *Journal of Nanomaterials.* **2016.**
23. Karaman, DS., et al. "Current Approaches for Exploration of Nanoparticles as animicrobial agents." Chap. 4 in Antibacterial agents, **2017.** 61-86.
24. Rutala, WA., David, JW., and HICPAC. guideline for Disinfection and Sterilization in Healthcare Facilities. CDC, **2008.**
25. Jensen, SO., et al. "Staphylococcus aureus dry-surface biofilms are not killed by sodium hypochlorite: implications for infection control." *Journal of Hospital Infection*, **2016.** 1-8.

26. Infection control unit. "Policies of surgical operations." In infection control policies and procedures index, 100-103. ismailia, egypt: suez canal university hospitals.
27. Mahmood, EL., and Doughari, JH., "Effect of Dettol on viability of some microorganisms associated with nosocomial infections." *African Journal of Biotechnology*, **2008.** 7(10): 1554-1562.
28. Alisaac, Ali., "In vitro Comparison of Five Different Hospital Disinfectants against Staphylococcus aureus (MSSA and MRSA) Collected from Healthy Volunteers."*Quinnipiac University*, **2011.** 1-70.
29. Samantha Storer, P., et al. "Disinfection with sodium hypochlorite in hospital environmental surfaces in the reduction of contamination and infection prevention a systematic review." *Journal of School of Nursing*, **2015.** 49(4): 675-681.
30. Ahoyo, TA., et al. "Staphylococcus sciuri outbreak at Tertiary Hospital in Benin." *Journal of Medical Microbiology and Dignosis*, **2013.** 2(3): 126.
31. Michelle, J., et al. "use of dialy disinfectant cleaner insted of dialy cleaner reduced hospital-acquired infection rates." *American Jornal of Infection Control*, **2015.** 141-146.
32. Alizadeh, Hamed., Mojtaba. Salouti., and Reza, Shapouri., "Bactericidal Effect of Silver Nanoparticles on Intramacrophage Brucella abortus 544." Jundishapur Journal of Microbiology, **2014.** 7(3).
33. Arnold, F., Sciortino, C., and Riede. K., New Associations With Pseudomonas Luteola Bacteremia: A Veteran With A History Of Tick Bites And A Trauma patient with pneumonia. The Internet Journal of Infectious Diseases. **2004.**
34. Barbut, Frederic., Menut, D., Verchaten, M., and Girou, E., "Comparison of the Efficacy of a Hydrogen Peroxide Dry-Mist Disinfection System and Sodium Hypochlorite Solution for Eradication of Clostridium difficile Spores." Infection Control and Hospital Epidemiology, **2009.** 507-514.
35. Carroll, KC., "The Streptococci, Enterococcci, and related organisms." Chap. 14 in Jawetz, Melnick & Adelberg's Medical Microbiology, by Geo F Brooks, Karen C Carrol, Janet C Butel, Stephen A Morse and Timothy A Mietzner, **2012.** 209-227.
36. Chaloupka, K., Yogeshkumar, Malam., and Alexander MS. "Nanosilver as a new generation of nanoproduct in biomedical applications." Trends in Microbiology (Cell press), **2010.** 28(100): 580-588.
37. Chan, MC., et al. "Applying ATP bioluminescence to design and evaluate a successful new intensive care unit cleaning program." *Journal of Hospital Infection*, **2015.** 344-346.
38. Charles river Research Models and Services. Pasteurella pneumotropica- technical sheet. Charles river laboratories international, inc. **2009.**

39. Chihab, W., Ahmed SA., and Mohamed, Amar., Chryseomonas luteola Identified as the Source of Serious Infections in a Moroccan University Hospital. *American Society of Microbiology.* **2004.**
40. Cirkovic, Ivana., et al. "Identification and Antimicrobial Susceptibility Testing of Staphylococcus vitulinus by the BD Phoenic Automated Microbiology System." *Current Microbiology,* **2008.** 57: 158-160.
41. Coenye, T., and Hans JN., "In vitro and in vivo model systems to study microbial biofilm formation." *journal of microbiological methods,* **2010.** 89-105.
42. Dancer, SJ., "Dos and don'ts for hospital cleaning." *Current Opinion in Infectious Diseases.* **2016.**
43. Fuente-Nunez, DL., "Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new theraputic stratigies." *Current Openion in Microbiology*, **2013.** 1-10.
44. Edwards-Jones, V., "the benefits of silver in hygiene, personal care and health care." *Letters in Applied Microbiology*, **2009.** 147-152.
45. El-Batal, A I, Bakry M Haroun, Ayman A Farrag, Ahmed Baraka, and Gharieb S El-sayyed. 2014 b. "synthesis of silver nanoparticles and incorporation with certin abtibiotic using gamma irradiation." British Journal of Pharmaceutical Research 4 (11): 1341-1363.
46. El-Batal, A I, M A Amin, Mona M K Shehata, and Merhan M A Hallol. 2014 a. "synthesis of silver nanoparticles by Bacillus stearothermophilus using gamma radiation and their antimicrobial activity." World Applied Science Journal 22 (1): 1-16.
47. El-Batal, Ahmed, I., et al. "Physiological Responses of two varieties of common Bean (Phaseolus vulgaris L.) to foliar application of silver nanoparticles." *Nanomaterials and Nanotechnology*, **2016.** 6(13): 1-16.
48. El-Batal, Ahmed Ibrahim., et al. "Impact of silver and selenium nanoparticles synthesized by gamma irradiation and their phsiological response on Early Blight disease of potato." *Journal of Chemical and Pharmaceutical Research*, **2016.** 8(4): 934-951.
49. Fraise, AP., "Decontamination of the environment and medical equipment in hospitals." In Russell, Hugo & Ayliffe's Principles and Practice of Disinfection, Preservation & Sterilization, by Adam P Fraise, Peter A Lambert and Jean-Yves Maillard, **2004.** 563-585..
50. Frebourg, NB., et al. Septicemia Due to Pasteurella pneumotropica: 16S rRNA Sequencing for diagnosis confirmation. *Journal of Clinical Microbiology.* **2002.**
51. Freeman, DJ., Falkiner, FR., and Keane, CT., "New method for detecting slime production by coagulase negative staphylococci." Journal of Clinical Pathology, **1989.** 42: 872-874.

52. Goodman, SD., et al. "Biofilm can be dispersed by focusing the immune system on a common family of bacterial nuceoid-associated proteins." **mucosal immunology**, **2011.** 625-637.
53. Hassan, A.,. "Evaluation of different detection methods of biofilm formation in the clinical isolates." *Brazilian Journal of Infectious Diseases,* **2011.** 15(4): 305-311.
54. Hong, Yi-Fan., et al. "Effect of NaCl and organic acids on antimicrobial activity of Chitosan." *Korean Journal of Microbiology and Technology,* **2014.** 413-416.
55. Igbinosa, EO., and Anthony IO., "Vibrio fluvialis: An unusual Enteric pathogen of increasing public health concern." *International Journal of Environmental Research and Public Health*, **2010.** 3628-3643.
56. Infection cotrol managment at ministry of health and population. 2008. "Policies of infection control in healthcare facilites." Egypt: ministry of health. **2014.** 95-98.
57. Inweregbu, Ken., Jayshree, Dave., and Alison, Pittard., "Nosocomial Infection." Continuing Education in Anaesthesia, Critical Care & Pain, **2005.** 14-17.
58. Janda, JM., and Sharon, LA., "The genus Aeromonas: Taxonomy, Pathogenicity, and Infection." *Clinical Microbiology Review*, **2010.** 35-73.
59. Kim, SA., and Rhee. MS., "synergistic antimicrobial activity of caprylic acid in combination with citric acid against both Escherichia col O157:H7 and indigenous microflora in carrot juice." Food Microbiology. **2005.**
60. Ko, KY., Mendonca, AF., and Ahn, DU., "influence of zinc, sodium bicarbonate, and citric acid on antimicrobial activity of ovotransferrin against Escherichia coli O157:H7 and Listeria monocytogenes in model system and ham." poultry science. **2009.**
61. Kwon, Young Jik., and Ae Jung Huh. ""Nanoantibiotics": A new paradigm for treating infectious disease using nanomaterials in the antibiotic resistance era." *Journal of Controlled Release*, **2011.** 128-145.
62. Martin, V., et al. "Characterization of Aerococcus viridans Isolates from Swine Clinical Specimens." *Journal of Clinical Microbiology*, **2007.** 3053-3057.
63. McDonnell, Gerald., and Denever, RA., "Antiseptics and Disinfectants: Activity, Action, and Resistance." Clinical Microbiology Reviews (*American Sociaty of microbiologh*), **1999.** 12(1): 147-179.
64. Mishra, Shyam Kumar., et al. "Detection of Biofilm Production and Antibiotic Resistance Pattern in Clinical Isolates From Indwelling Medical Devices." *Current Microbiology*, **2014.** 128-134.
65. Nase, Rasha., Hala, A., and Hussein, H., "Biofilm formation and presence of icaAD gene in clinical isolates of staphylococci." *the Egyptian journal of Medical Human Genetics*, **2012.** 269-274.

66. National Center for Emerging and Zoonotic Infectious Diseases. basic infection control and prevention plan for out patient oncology settings. center of disease control and prevention. **2011.**

67. Nemegharie, S., et al. "The ecological importance of the Staphylococcus sciuri species group as a reservoir for resistance and virulence genes." Vetrinary Microbiology. **2014.**

68. Oliveira, A., and Maria LC., "Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci." BMC Research Notes. **2010.**

69. Otter, JA., Saber, Y., and Gary LF., "The role played by contaminated surfaces in the transmission of nosocomial pathogens." *Infection Control And Hospital Epidemiology* (unviversity of chicago press), **2011.** 32(7): 687-699.

70. Park, GW., et al. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Edited by D W Schaffner. *Applied and Environmental Microbiology* (American Society for Microbiology), **2015.** 81 (17): 5987-5992.

71. Park, JH., et al. "Superior Vena Cava Syndrome Caused By Aerococcus viridans Para-Aortic Abscess After Heart Transplantation." *Transplantation*, **2002.** 74(10): 1475-1476.

72. Ramamurthy, T., Goutam, C., Guruzaja, PP., and Sumio, S., "Vibrio fluvialis: an emerging human pathogen." Frontiers in Microbiology. **2014.**

73. Randrianirina, F., et al. "In vitro activities of 18 antimicrobial agents against Staphylococcus aureus isolates from the Institut Pasteur of Madagascar." *Annals of Clinical Microbiology and Antimicrobial*, **2007.** 1-5.

74. Bose, S., Khodke, M., and Basak, S., "Detection of biofilm producing Staphylococci: Need of the hour." *Journal of Clinical and Diagnostic Research*, **2009.** 3(6): 1915-1920.

75. Singh, P., et al. "The development of a green approach for the biosynthesis of silver and gold nanoparticles by using Panax ginseng root extract, and their biological applications." International Journal of Nanomedicine, **2015.** 1-8.

76. David, A., et al. "Comparative Global Transcription Analysis Of Sodium Hypochlorite, Peracetic Acid, And Hydrogen Peroxide On Pseudomonas Aeruginosa." *Applied Microbial Biotechnology*, **2007.** 1093-1105.

77. Stepanovic, S., et al. "quantification of biofilm in microtitre plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci." *APMIS***, 2007.** 891-899.

78. Stepanovic, S., et al. "A comparative evaluation of phenotypic and molecular methods in the identification of members of the Staphylococcus sciuri group." Systemic and Applied Microbiology, **2005.** 353-357.

79. Stepanovic, et al. "Isolation of Members of the Staphylococcus sciuri Group from Urine and Their Relationship to Urinary Tract Infections." *Journal of Clinical Microbiology*, **2003.** 5262-5264.

80. Tena, D., and Cristina, F., "Pseudomonas oryzihabitance: an unusual cause of skin and soft tissue infections." *Infectious Diseases*, **2015.** 820-824.
81. Tewazi, R., et al. "Isolation of Aeromonas salmonicida from human blood sample: a case report." Journal of Clinical and Diagnostic Researches, **2014.** 139-140.
82. Patricia, M., "Staphylococcus, Micrococcus, and Similar Organisms." Chap. 14 in Baily and Scott's Diagnostic Microbiology, by Patricia M Tille, **2014.** 233.
83. Patricia TM. "Streptococcus, Enterococcus, and Similar Organisms." Chap. 15 in Baily and Scott's Diagnostics Microbiology, by Patricia M Tille, **2014.** 247-264.
84. Tirumali, RS., *Disinfectants and Antiseptics*. **2018.** 30: USP30- NF25,
85. Torok, ME., Fiona, JC., and Moran, ED., Oxford Handbook of *Infectious Diseases and Microbiology*. **2009.**
86. Margereet, VC., "The Healthcare Workers as a Source of Tranmission." Chap. 11 in A Guide to Infection Control in the Hospital, edited by Gonzalo M. l Bearman, Michael Stevens, Michael B Edmond and Richard P Wenzel, **2014.** 57-67.
87. Barbara, V., et al. "Bacterial extracellular polysaccharides involved in biofilm formation." Molecules, **2009.** 14: 2535-2554.
88. Zhen, W., et al. "in vitro evaluation of six chemical agents on smooth Brucella melitensis strain." Annals of Clinical Microbiology and Antimicrobials. **2015.**
89. Webster, JA., et al. "Identification of the Staphylococcus sciuri species group with EcoRI fragments containing rRNA sequences and description of Staphylococcus vitulnus sp." *International Journal Of Systematic Bacteriology*, **1994.** 44 (3): 454-460.
90. Weleh, K., Yanling, C., and Maria, S., "*A method for quntitative determination of biofilm viability." Journal of Functional Biomaterials*, **2012.** 3: 418-431.
91. Richard, WP., "Importance of Infection Control." In A Guide to Infection Control in the Hospital, 1. *The International Society for Infectious Diseases*. **2014.**
92. Kwang-Sook, W., et al. "Outbreak of Pseudomonas Oryzihabitans Pseudobacteremia Related to Contaminated Equipment in an Emergency Room of a Tertiary Hospital in Korea." Infection and Chemotherapy Journal, **2014.** 42-44.
93. Wood, T., Seok, HH., and Qun, M., "Engineering biofilm formation and dispersal." *Applied microbiology* (Cell press), **2011.** 29 (2): 87-94.

94. Hong, X., et al. "A randomized, double-blind comparison of the effectiveness of environmental cleaning between infection control professionals and environmental service workers." American Journal of Infection Control. **2015.**