

Assessment of Disinfectant Performance Procedures Applied in Small sector of Egyptian poultry farms

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Abstract

Three different disinfection programs were applied in the three poultry (pines) houses in faculty of veterinary medicine at Cairo University which simulate small scale Egypt poultry farm sector using available traditional materials that easily obtained from Egyptian market. In the cleaning process we used surfactant, foam technique and 5 % chlorinated surfactant in Program A, B, C respectively and in the disinfection process we used quick lime mixed with cresol, quick lime mixed with Calcium hypochlorite and quick lime mixed with cresol and Calcium hypochlorite in program A, B, C respectively. The disinfection programs take three days through these steps which are whipping (Dry cleaning), applying water with high pressure directly (Wet cleaning), applying the detergent with brushing directly twice, after rinsing with water immediately at the first day. At the second day, applying the disinfectant was done. At the third day, the house is left to dry. Swabs were taken before and after each application. Fifteen swabs were taken from (walls, floor and roof) 5 swabs per each before and after each application. The log mean of the average of each five was calculated. It is concluded that the three programs were effective and successful as all of them achieved the reduction which is as standard optimization for the disinfection process. The use of the foam technique in cleaning process produced an excellent result; also the addition of chlorine to the surfactant achieved an observable reduction in microbial viability. Local Egyptian market disinfectant products present and produce in Egyptian market should be evaluated periodically as they may be devalued or less in their active ingredients regarding to the labeled ingredients.

Key words: poultry - cleaning – disinfection- quick lime - cresol- surfactant- Calcium hypochlorite - total colony count.

Introduction

Commercial poultry production is rapidly expanding in all over the world and in Egypt to meet the needs of the increasing population. Small scale poultry farming has a major share in our Egyptian market. Due to crises of unemployment and rumors of high profit gains in short time in poultry production some recently graduated degrees and ungraduated begin to rear poultry in any area near in their locality. It may be apartment or room in their house or roof of their houses. Claiming that they are expertise stockman the number they rear in this facility may range from (100 to 3000). Accordingly; this sector threatened other part of Egyptian poultry industry, through direct or indirect shedding, the causative agent to human and poultry industry population. Recent outbreaks of some viral and bacterial diseases in poultry industry among bring to the forefront the importance of proper cleaning and disinfection (C&D) practices for poultry farms. Sanitation is the most important parts of commercial poultry production &

management which is usually neglected. It means cleanliness at the farm to prevent outbreak of disease. Sanitation starts with the quality of cleanliness, while disinfection refers to the reduction of contamination.

Reducing the load of pathogens in the environment of the flock will decrease the risk of disease (Jeffrey 2005). Various deficiencies in (C&D) procedures may induce a chain of infections which will not break from one stock to another (Kaskova et al., 2007). As an improper cleaning can actually do more harm than good. If done properly, a good cleaning can remove 90% of the pathogens (Gordon and Morishite, 2007). The last step in a (C&D) program is the actual disinfection process was done. This involves the use of disinfectants that will reduce or kill the pathogens. There are several types of disinfectants, and the chosen disinfectant should be effective against the disease agents that is being targeted (Koulikovskii, 1984).

Surfactant exhibit properties of tension lowering and this is why they are often labeled according to their main use such as: detergent, wetting agent, dispersant, emulsifier, foaming agent, bactericide, corrosion inhibitor, antistatic agent. Although Quaternary ammonium compound (QAC) has limited germicidal range not sporicidal, effective against vegetative bacteria, fungi and viruses (Gamage, 2003). They are non-influencing, non-corrosive and have low toxicity and best cleansing agent. Use of foams result from QAC produces excellent results against different types of bacteria. The advantages of using foam techniques are dissolving grease, Biofilm and crust on almost any washable surface, non-abrasive, beside fast-acting foam won't scratch the surface. The foam substances had specific effects which could be used not only for cleaning surfaces from common dirt, destroying toxic substances, and or focal disinfection. (Shimaa 2014) Cresols are coal-tar derivatives. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses. They are the active ingredients in some house hold disinfectants. Their common uses in commercial animal production unit include hatchery equipment, sanitation. Cresol is relatively inexpensive and efficient as a disinfectant. It is not readily soluble in water and hot water should be used for preparing solutions. Cresol preparations such as "Lysol" are mixtures of cresol with soap to form more readily soluble solutions for easier application. (Gamage,2003)

Hypochlorite's are the most widely used of the chlorine disinfectants and are available in a liquid (e.g. sodium hypochlorite) or solid (e.g. calcium hypochlorite, sodium dichloroisocyanurate) form. Hypochlorite has long been recognized as powerful and efficient disinfectants. The sodium and potassium salts have not been generally employed on a large scale because of their relatively high cost, but calcium hypochlorite has been rather extensively used. Calcium

Materials and Methods

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hypochlorite or chloride of lime, also known as "bleaching powder," contains from 30 to 95 % of available chlorine.

Lime (calcium oxide, quicklime, QL) is one of the least expensive disinfectants and is reasonably good for use around livestock. Powdered lime may be scattered about yards or lots or swept over concrete floors for general disinfection. Stringfellow al 2010 hypothesized that steam and QL would reduce Salmonella Typhimurium in broiler litter. On the other hand, complete dryness of a surface lead to desiccation of micro-organism which has a lethal effect on their number and viability (Xie. X et al, 2006).The final step of ensuring a proper clean-up is having the wet areas of the building dried quickly. If the building is not dried properly, the excess moisture can result in bacteria multiplying to higher

level than seen before cleaning

(Morishita&Jordan2002).

Three programs (A, B and C) are undertaken in this study (table 1, 2) to assess the efficacy of three different programs and the procedures of the program. In program (A) we used surfactant and quick lime mixed with cresol, in program (B) we used the foaming technique and quick lime mixed with chlorine, in program (C) we used chlorinated surfactant and quick lime mixed with Calcium hypochlorite and cresol in the cleaning and disinfection process respectively. Also determine active chemical material in each disinfectant we used. In order to achieve correct concentration to applied.

Therefore, this work aimed to apply simple and effective procedure for (C&D) poultry house using available traditional disinfectants and detergents material that easily obtained from the Egyptian market. Therefore, this work conducted to evaluate the efficiency of each step of (C&D) process, this efficiency lead to make the necessity for evaluation of the disinfectant which used in Egyptian markets and their role in reducing the bacterial load in commercial poultry farms.

Management Unit (PMU), Ministry of higher Education; Egypt

1-House & programs description

Three poultry (pines) houses in Faculty of Veterinary Medicine at Cairo University which simulate small scale Egypt poultry

farm sector, three different (C&D) programs in 3 experimental poultry houses (pines) each of Them (4×4 m²) with

height 3meter were used as showed in table (1)

Table (1) The different disinfection programs in the three experimental poultry pines

Practice	Program A	Program B	Program C
Twice Detergent subject	Surfactant 1/2 L Detergent+ 9.5 L water)	Surfactant (1/2 L Detergent+ 9.5 L water) + blowing air through blower in solution till foam are produced in sufficient amount	0.1 kg of Bleaching powder + surfactant (1/2 L Detergent+ 9.4 L water)
Disinfectant agent	One Kg of Quick lime +0.3 L of cresol+8.7 L water	One kg of Quick lime + 0.1 kg of bleaching powder +8.9 L water	1 Kg of Quick lime + 0.3 L of cresol + 0.1 kg of bleaching powder + 8.6 L water

2-Procedure of (C&D)

2.1 First day:

- 1- Whipping and removal of all organic matter immediately. (Dry cleaning)
- 2- Applying water with high pressure directly. (Wet cleaning)
- 3- Applying the detergent with brushing.
- 4- Applying high pressure rinsing with water immediately.

3- Chemical analysis of (C&D

Agent)

3.1-Determination of Chloride in Commercial Ca hypochlorite (bleaching powder)

by British pharmacopeia 2012:

By dissolving 0.28g of the powder in 100 ml of water and carries out the complex metric titration of Calcium. One mL of 0.1 M sodium edetate is equivalent to 14.7 mg of CaCl₂, 2H₂O. Determination of chloride is according to the molecular weight.

3.2-Determination of Commercial quick lime

According to (Vogel's 1989):

Ammonium oxalate precipitates calcium quantitatively as calcium oxalate. An excess of oxalate overcome the adverse effects of magnesium. Optimum crystal formation and minimum occlusion are obtained only when the pH is brought slowly to the desired value. This is accomplished in two stages, with intervening digestion to promote seed crystal formation. The precipitated Ca oxalate is dissolved in acid and titrated with Permanganate. The amount of Permanganate required to oxidize the

5- Applying the detergent with brushing directly second time.

6- Rinsing & leave to dry.

2.2 Second day

7- Applying the disinfectant.

2.3 Third day

8- Leave house to dry.

oxalate is proportional the amount of calcium.

3.3 Determination of carbolic acid (cresolic acid)

According to (Indian standard based on Bromide-bromate solution method 2000): The method is based on the reaction between phenol and bromine from standard bromide-bromate solution and titrate the contents with standard sodium thiosulphate solution.

3.4 Adjustment of the disinfectant concentration

According to our chemical analysis, readjust the commercial Calcium Hypochlorite, Cresolic acid and quick lime form labeled concentration to estimated concentration to obtained applied desired contraction that applied in our experiment.

According to our chemical analysis, the actual concentration of these active ingredients of the disinfectants were varied dramatically from that labeled; So adjustment of the concentration of our disinfectant was done in order to get the accurate concentration that applied in our experiment.

Disinfectants agents which used in our study and its chemical analysis:**Table (2)** The disinfectant agents which used in the three experimental poultry programs

Disinfectant	Concentration		Manufacture
	Labeled	Estimated	
Commercial Calcium hypochlorite (Chlorine powder)	84% conc.	59%.	Unknown
Prii ^R :	Surfactant 15-30% Anionic surfactant, less than 5 % nonionic surfactant, preservatives, enzymes and perfumes.		Henkel factory production (Made in Egypt)
Cresolic acid	30%	17% conc.	Ramdan factory production
Commercial quick lime	CaO 84.8%	60 %	Unknown

4-Microbiological examination:

According to (A.P.H.A., 1984) Swabs were collected before and after each step. Fifteen swabs were taken from (walls, floor and roof) 5 swabs per each and the average of each five was calculated and the log mean was obtained. Swabs from walls, floor and roof were collected with sterile cotton normal saline solution that were used for obtaining samples from outlined squares using wire template 10 X 10 cm, according to (Collins et al 1991). These swabs were received into sterile cotton plugged test tubes containing 5 ml of sterile saline solution (8.5g sodium chloride in 1000 ml. distilled water). After disinfectants or detergents application and definite contact time, swabs were taken into neutralizer tubes to stop the action of disinfectants.

The neutralizer used was prepared according to (Horejsa and Günter 2010): Combination of 3% Tween 80

2.1 First day:

- 1- Before applications.
- 2- After whipping and removable of all organic matter immediately. (Dry cleaning)
- 3- After applying water with high pressure directly. (Wet cleaning)
- 4- After applying the detergent with brushing directly.
- 5- After rinsing with water immediately.

Results

(polysorbate 80) (Mp Biomedicals), 0.3 Lethcine (Fisher chemicals), 1% Histidine (Fisher chemicals), 0.5% Sodium thiosulphate (Fisher chemicals), 3% Saponine (Fisher chemicals).

The used test tubes were transferred to laboratory in an ice box as soon as possible then ten-fold serial dilution were applied and duplicated tryptic soya agar plates were inoculated each with 0.1 ml from each dilution using spread plate according to surface disinfection tests according to (European Committee for standardization 2002 European standard EN13697). Air samples were collected by settling plate count technique according to (Soucy et al., 1983) using Tryptic Soya Agar (TSA). The inoculated plates were incubated at 37°C for 24 hours before counting. The plates giving 30-300 colonies were used for the calculation of the average total colony count.

- 6- After applying the detergent with brushing directly second time

2.2 Second day

- 7- After 24 hours from rinsing.
- 8- After an hour from applying the disinfectant.

2.3 Third day

- 9- After 24 hours from applying the disinfectants

Table (3) Performance of disinfecting programs on walls

Procedure	Log of total colony count		
	Program A	Program B	Program C
Initial Bacterial Count	7.6	8	8
Dry Cleaning	7.3	8	7.3
Applying High Pressure Cleaning	7	6.3	6.3
Detergent With Brushing first time	5.3	4.6	3.47
Rinsing With Water Immediately	5.2	4.5	3.4
Detergent With Brushing second time	2.47	3	3.3
24 Hours After Rinsing	2.3	2	1.3
After An Hour From Applying The Disinfectant	1	1	1
24 Hours After Applying the Disinfectants	0	0	0

Figure 1 Performance of disinfecting programs on walls

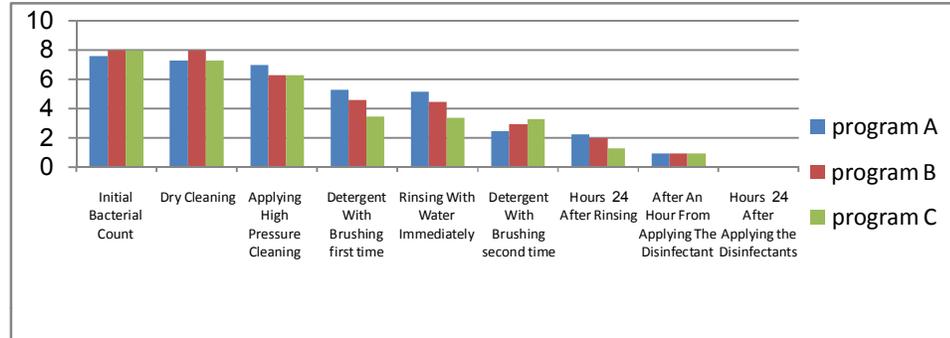


Table (4) Performance of disinfecting programs on floor

Procedure	Log of total colony count		
	Program A	Program B	Program C
Initial Bacterial Count	7.6	8	8.6
Dry Cleaning	7	7.3	8
Applying High Pressure Cleaning	6	6.84	5.84
Detergent With Brushing First time	5.9	4.69	4.47
Rinsing With Water Immediately	5.84	4.5	4.4
Detergent With Brushing second time	2.3	3	4.3
24 Hours After Rinsing	2	2	2
After An Hour From Applying The Disinfectant	1.3	1	1
24 Hours After Applying the Disinfectants	0	0	0

Figure 2 Performance of disinfecting programs on floor

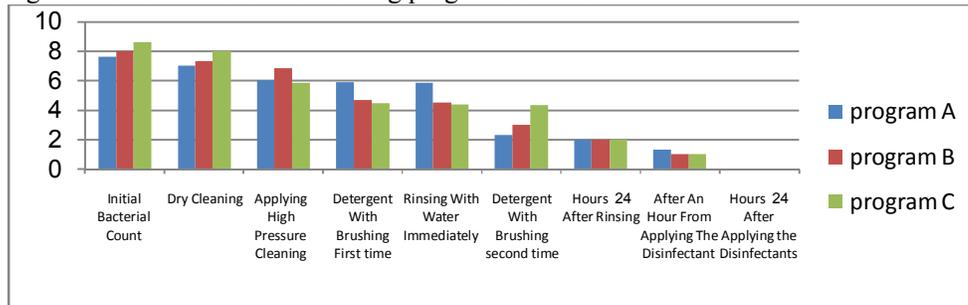


Table (5) Performance of disinfecting programs on roof

Procedure	Log of total colony count		
	Program A	Program B	Program C
Initial Bacterial Count	6.3	7.3	7
Dry Cleaning	6.3	7.3	5.47
Applying High Pressure Cleaning	5	5.3	5
Detergent With Brushing First Time	4.9	3.9	3
Rinsing With Water Immediately	4.47	3.7	2.9
Detergent With Brushing Second Time	1.3	2	2.5
24 Hours After Rinsing	1.2	1	1.47
After An Hour From Applying The Disinfectant	1	1	1
24 Hours After Applying the Disinfectants	0	0	0

Figure 3 Performance of disinfecting programs on roof

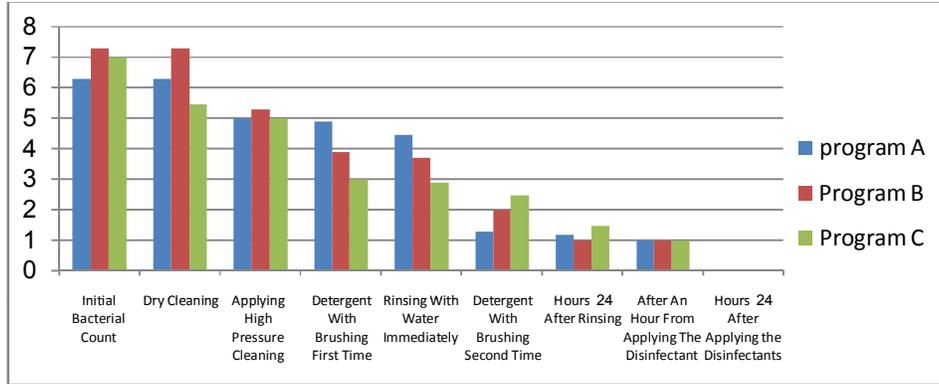
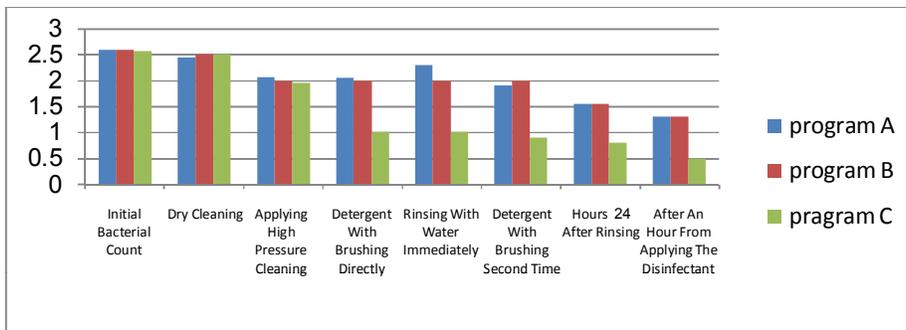


Table (6) Performance of disinfecting programs on air

Procedure	Log of total colony count		
	Program A	Program B	Program C
Initial Bacterial Count	2.6	2.6	2.57
Dry Cleaning	2.45	2.5	2.5
Applying High Pressure Cleaning	2.07	2	1.95
Detergent With Brushing Directly	2.05	2	1.01
Rinsing With Water Immediately	2.3	2	1
Detergent With Brushing Second Time	1.9	2	0.9
24 Hours After Rinsing	1.55	1.55	0.8
After An Hour From Applying The Disinfectant	1.3	1.3	0.5
24 Hours After Applying the Disinfectants	1	1	0.3

Figure 4 Performance of disinfecting programs on air



Discussion

Choosing an effective disinfectant regime is appropriate to the hygiene requirements in commercial poultry management. In this context, consideration of the antimicrobial efficacy of different disinfection procedures is important decision-making.

From table (2) chemical determination of active ingredient in each material we used help us to precise a correct concentration of disinfectant that used in all three programs the actual concentration of these chemical are varied dramatically from that labeled. As chlorine powdered was labeled 84% active chlorine but actual concentration of active ingredient was 59% while cresol was labeled 30% but the actual active ingredient was 17%, regarding too quick lime it was actually 60% Cao while it was labeled 85%. So, adjustment the concentration of our disinfectant was done in order to get accurate results.

From table (3,4,5&6) and Fig (1,2,3&4) it is clearly noticed that dry cleaning (whipping) showed the highest count in all of the three programs as it is no noticeable log reduction happened in the all of the programs from initial count as the process is the same in the three applied programs, this process is called dry cleaning. The importance of this process is to remove the organic matter which hinders the disinfectant action. This result agrees with Sainsbury 2000 and Payne et al 2005 whose mentioned that the presence of organic matter influence disinfection.

Wet cleaning practice is to apply the water with high pressure. However, it is more important to have pressure washers with the proper pressure to ensure all the organic materials are removed from the facilities. The results of average log of the total bacterial count of walls were 7, 6.3, 6.3, floor 6, 6.84, 5.84 roof 5, 5.3, 5 air 2.029, 2.07, 1.95 in program A, program B and program C respectively. The log total bacterial reduction is more improved but still high as the average log reduction of the total bacterial of walls were 0.6, 1.7,1.7, floor 1.6, 1.6, 2.76, roof 1.3, 2, 2 air 0.44, 0.53, 0.62 in program A, program B and program C respectively. This result agrees with Davis and Wray 2002, Miguel et al 2001 and Ledouxet al 2005 who mentioned that the intention of disinfectant

programs in poultry facilities is to reduce the populations of diseased bacteria and if we do the disinfection process without cleaning the disinfection process will be compromised.

The next technique is to use the detergent with brushing first time in the three programs, twice the results of the average log of the total bacterial count of walls were 5.3, 4.6, 3.4, floor 6,4,6,4.4 roof 5,3,9,3 and air 1.95,2.05, 2.1 in program A, program B and program C respectively. The results of average log reduction of the total bacterial count of walls were 7, 6.3, 6.3, floor 6, 6.84, 5.84 roof 5, 5.3, 5 air 2.029, 2.07, 1.95 in program A, program B and program C respectively. The results of average log reduction of the total bacterial count from the first step of walls were 2.3, 3.4, 4.5, floor 1.7, 3.31, 4.13, roof 1.4, 3.4, 2 air 0.52, 0.55, 1.56, in program A, program B and program C respectively. Brushing is leading to increase the effectiveness of the detergent. It also helps to remove the stacked organic matter if it is found. After using the detergent, we rinsed the three pines immediately and take the samples. The results of the average log of the total bacterial count were walls were 5.3, 4.69, 5.47, floor 5.84, 5, 5.3 and roof 4.4, 4.3, 5.4 air 2.07,2.03,2.3 respectively in program A, program B and program C. The results of average log reduction of the total bacterial count of walls were 2.4, 3.5, 4.6, floor 1.76, 3.5, 4.2, roof 1.83, 4, 3.6, air 0.97, 0.567, 1.57, in program A, program B and program C respectively.

Detergent with brushing for the second time, the total bacterial count dramatically decreased. As the result were walls 2.4, 3, 3.7, floor 2.3, 3, 4.7 roof 1.3, 2, 3.3 air 1.85, 1.9, 2.1 in program A, program B and program C respectively. The results of average log reduction of the total bacterial count of walls were 5.13, 5, 4.7, floor 5.3, 5, 4.3 roof 1.83, 5.3, 4.1 air 0.97, 0.7, 1.67 in program A, program B and program C respectively. These result agree with Ruano et al 2001 found that the contact time which is recommended by the manufacturer should be increased in order to maintain their efficacy. Moustafa et al 2009 mentioned that when organic matter is present, longer contact time were needed to demonstrate the effectiveness of

disinfectants in the examined hatchery surfaces.

In program B we used the foam technique; the use of foam in cleaning in house B produced excellent result when compared with house A and C, this result agreed with that obtained by (Severa et al 2005) who mentioned that the use of foams in disinfection is universally advantages and means a great advance in disinfection technology, it produces excellent results against different types of bacteria. The advantages of using foam techniques which is dissolves grease, soap film and

Conclusion

Chemical determination of active ingredient in each disinfectant is a must in order to practice correct concentration you have. An effective cleaning and disinfection program is a crucial step in every poultry-biosecurity program, Cleaning and disinfection should be taken together as they tend to be recommended as a single phrase without differentiation. Every process has a role to achieve our goal. Whatever the disinfectant you choose, the application and the efficiency of the steps is the point of difference. The use of foam in cleaning process produces an excellent result in comparison with other methods.

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- scum on virtually any washable surface, non-abrasive, fast-acting foam won't scratch the surface
- In program C we use the chlorinated surfactant, the addition of chlorine increases the effectiveness of the cleansing procedures, as the chlorine is the most commonly manufactured chemicals for the disinfection, it is soluble with the surfactant.
- The three programs are effective and successful as all of them achieve the five log reduction which is the standard optimization for the disinfection process. Disinfection program using quick lime and chlorine powder should be recommended.
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الملخص العربي

طبقت ثلاثة برامج التطهير مختلفة في ثلاثة عنابر لتربيته الدواجن في كلية الطب البيطري في جامعة القاهرة التي تحاكي نطاق صغير بالقطاع الزراعي في مصر لتربيته الدواجن باستخدام المواد التقليدية المتاحة التي يمكن الحصول عليها بسهولة من السوق المصري. في عملية التنظيف استخدمنا الصابون السائل، وتقنية الرغوة و 5% كلور مختلطه بالصابون السائل في برنامج C،B،A على التوالي وفي عملية التطهير استخدمنا الجير الحي مختلط مع الكريسول، الجير الحي مختلط مع هيبوكلوريت الكالسيوم الجير الحي مختلط مع كريسول والكالسيوم هيبوكلوريت في برنامج C،B،A على التوالي. استغرق برنامج التطهير مدة ثلاثة ايام من خلال هذه الخطوات والتي هي الكنس (ادوات التنظيف الجاف)، وتطبيق المياه مع ارتفاع ضغط مباشرة (التنظيف الرطب)، وتطبيق المنظفات مع تنظيف الأسنان بالفرشاة مباشرة مرتين ثم الشطف بالماء فوراً في اليوم الأول. في اليوم الثاني، تم استخدام مطهر. في اليوم الثالث، يترك العنبر لييجف. أخذت مسحات قبل وبعد كل تطبيق. أخذت خمسة عشر مسحة من (الجدران والأرضيات والسقف) 5 مسحات لكل وتم حساب المتوسط. وخلصت الدراسة إلى أن البرامج الثلاثة كانت فعالة وناجحة كما كل منهم حقق الحد الذي هو الأمثل القياسي لعملية التطهير. استخدام تقنية رغوة في عملية التنظيف نتج عنه نتيجة ممتازة، أيضاً إضافة الكلور إلى الصابون السائل حقق انخفاضاً ملحوظاً في العد البكتيري وأن المنتجات في السوق المصرية المحلية يجب أن يتم عمل تقييم دوري لها لأنها قد تكون تحتوي على قيمة أقل في المواد الفعالة بها.