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To Check Efficacy of Different Disinfectants by Kelsey Sykes Method

Singh H[1*], *P* Singh[1] and *A* Sharma[2]

[1]Départment of Médical Laboratory Sciences, Lovely Professional University, Punjab, India
[2]Départment of Lab Medicine, Fortis Hospital, Mohali, India

*Corresponding author: harpreet1.singh@lpu.co.in

ABSTRACT
The incidence of hospital and community acquired infections has globally increased worldwide. The concentration of the disinfectants is important to give its higher efficacy against pathogens or microorganisms. Microorganisms are part of or environment cause major or severe diseases to reduce the risk of these diseases we generally clean surrounding by using cleaning agents like soaps, antiseptics or disinfectants. So to check weather these disinfectants are performs there work against specific bacteria or microorganism we performed this test to check the effectiveness of disinfectants against specific microorganism and fungus. Five bacterial types that are frequently acquired in hospitals from which two are gram positive organism, two are gram negative organism and single strain of fungus '(Pseudomonas *aeruginossa*, Klebsiella *pneumoniae*, Staphylococcus *aureus (MRSA)*, Acinetobacter *baumannii*, Candida *tropicalis*) (clinical isolates) were examined for their susceptibility against 3 commonly used disinfectants hospitals Chlorine releasing agent, Quaternary ammonium compound and Aldehyde free disinfectant). The turbidity in the nutrient broth and the growth in culture media showed that all three disinfectants had lowered the microbial growth of every clinical strain. In general, the reference strains have lower growth and turbidity than the clinical strains. When combined, our results demonstrated that every product we examined had a high disinfection killing rate against microorganisms from various sources, indicating the excellent caliber of these goods. In response to rising disinfectant usage, hand sanitizer production has expanded to keep up with demand. Normal flora or microorganisms are part of our body in everyday life in which some are necessary some are unnecessary and can cause major or severe diseases to avoid from these diseases we generally clean our self and surrounding by using cleaning agents like soaps, antiseptics or disinfectants. So, to check weather these sterilizing agents are performed their work against specific bacteria or microorganism we performed this test to check the effectiveness of disinfectants against specific microorganism and fungus.

1 INTRODUCTION

In this era, the rate of transmission of hospital acquired infections, diseases are increasing in hospitals or person to person globally. The Centers for Disease Control and Prevention (CDC) estimate that over 2 million individuals globally get nosocomial infections each year, which lead to roughly 90,000 fatalities. There are different types of patients in hospital environment which are suffering from different types of diseases and can transmit through water droplets and hands.

As per the 2018 data by Tri-Country Healthcare, over 80% of human illnesses can be spread through contact. The total number of microflora on our body is similar to number of human cells Staphylococcus *aureus* which is a residential microflora of skin [1]. There are large number of microflora on the skin anywhere else on the body. Mostly skin microbiota is found on the upper layer of the skin in the hair follicles. So, bacteria, viruses are very harmful if it is not a normal flora of our body. Staphylococcus *aureus* is a normal flora of our skin or nose but it is entered our skin it might be pathogenic for us and cause severe infection such as pneumonia or blood stream infections in the body

The most common resident flora are present on skin Staphylococcus *aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), and Enterococcus *faecalis* (*E. faecalis*) in the environment Escherichia *coli (E. coli)*, Salmonella *enteritidis* (*S. enteritidis*) *and* Pseudomonas *aeruginossa* (*P. aeruginossa*) are also present in the hospital which can cause nosocomial infection[5].

There are different types of microorganisms in hospital environment that can cause urinary tract infection blood stream infection or respiratory infection. The mainly reported cases of harmful pathogens are of (Klebsiella *pneumoniae*, *E. coli*, *P. aeruginossa*, and Acinetobacter *baumannii*), and Gram-positive bacteria (Staphylococcus *aureus (MRSA)* and fungi Candida *tropicalis* (*C*. tropicali*s*). Disinfectants of environmental surface is extremely imparted to recite risk of infections occurring out from these microorganisms.

There are many of disinfectants used to disinfect microorganisms which are as follows :-

- **Chemical Disinfectants** – chemical disinfectants used are alcohol, Chlorine and chlorine compounds, Formaldehyde, Glutaraldehyde, Hydrogen peroxide, Iodophors, Ortho-phthalaldehyde (OPA), Peracetic acid, Peracetic acid and hydrogen peroxide, Phenolics, Quaternary ammonium compounds
- **Miscellaneous Inactivating Agents** – The inactivating agents used are other germicides, Metals as microbicides, ultraviolet radiation and pasteurization

In this study three types of disinfectants are used:-

1. Chlorine-releasing agents (*10% Sodium Hypochlorite)*
2. Aldehyde free disinfectant
3. Quaternary Ammonium disinfectant

2 Material and Methods

Five bacterial strains of commonly hospital acquired infections from which two are gram positive organism, two are gram negative organism and single strain of fungus '(Pseudomonas *aeruginossa*, Escherichia *coli*, Klebsiella *pneumoniae*, Staphylococcus *aureus(MRSA)*, Acinetobacter *baumannii* , Candida *tropicalis*) (clinical isolates) were examined for their susceptibility against 3 commonly used disinfectants hospitals Chlorine releasing disinfectant , Quaternary ammonium disinfectant and Aldehyde free disinfectant. The microbial growth of all clinical strains was shown to be decreased by all three disinfectants, as demonstrated by the growth in culture media and the turbidity in nutritional broth[48]. In general, the reference strains have lower growth and turbidity than the clinical strains. When combined, our results demonstrated that all of the tested products had high disinfection killing rates against microorganisms of various origins, pointing to both the high caliber of these disinfectants and the effective surveillance methods used by Indian local government agencies.

EQUIPMENTS USED: Autoclave, Incubator, Bio-Safety Cabinet, Hot air oven

MATERIALS USED: Glass Wares, Micropipette, Glass test tubes, Measuring cylinder

Mackonkey agar, Nutrient broth

EXPERIMENTAL SETUP:

The experimental setup required for this project was organized at Clinical Microbiology Department of Lab Medicine, Fortis Mohali. All the procedures were conducted under sterile conditions inside bio-safety cabinet.

Bacterial clinical isolates - The clinical isolates of Klebsiella *pneumonia, Methaicillin-resistant* Staphylococcuss *aureus (MRSA),* Acintetobacter *baumannii,* Pseudomonas *aeruginossa and* Candida *tropicalis*

These were employed in the investigation to evaluate the efficacy of various disinfection agents. Hospitalized patients' clinical stains were isolated. The VITEK 2 system (bioMérieux) was utilized to identify the isolates.

1 By using Chlorine releasing disinfectant

Firstly concentration is converted into 10 % to 1 % as per usage in hospitals and laboratories. 10ml of 10% Sodium Hypochlorite and added into 100ml of distilled water and final volume of 1% Sodium Hypochlorite is ready .

By measuring the optical density of 5 different microorganisms suspension with help of VITEK DENSICHEK instrument is an accessory intended for use with the VITEK 2 Systems.

Table 1: Optical density of clinical isolates for Chlorine releasing disinfectant

Microorganisms	Optical density (McF)
S.*aureus* (MRSA)	1.02McF
K.*pneumoniae*	1.06McF
A.*baumannii*	1.03McF
P.*aeruginossa*	1.01McF
C.*tropicalis*	1.04McF

Now microbial suspension of positive control of 3ml of 5 different microorganism is ready.

3ml of 1% Sodium Hypochlorite is added into a 5 different glass test tube by labeling the name of micro organisms we use in this test (S. *aureus* (MRSA) , K. *pneumoniae*, A.*baumannii*, P.*aeruginossa*, C.*tropicalis*)

1 ml of microbial load is added into each of test tube having pure form of 3 ml 1%Sodium Hypochlorite by using micropipette and mix well.

- After 5 minutes of incubation : 20 µl with micropipette of sample and streak it into 1 mackonkey agar labeled as Klebsiella pneumoniae 5 min plate and another 20µl with micropipette pass in 5 ml nutrient broth labeled as Klebsiella pneumoniae 5 min and also 20 µl of sample from microbial suspension Klebsiella pneumoniae and added into mackonkey agar plate with nutrient broth tube labeled as positive control of Klebsiella pneumonia.
- After 10 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Klebsiella pneumoniae 5 min plate and another 20µl with micropipette pass in 5 ml nutrient broth labeled as Klebsiella pneumoniae 10 min.
- After 15 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Klebsiella pneumoniae 5 min plate and another 20µl passed in 5 ml nutrient broth labeled as Klebsiella pneumoniae 15 min. Same protocol followed with another four organism with this 0.4% Alliance R82

2. By using Quaternary ammonium disinfectant

Firstly concentration of final volume was made to 0.4% as per usage in hospitals and laboratories. 80µl of Quaternary ammonium disinfectant and 20ml of distilled water is added and final volume of 0.4% Alliance R82 was made. By measuring the optical density of 5 different microorganisms' suspension with help of VITEK DENSICHEK instrument is an accessory intended for use with the VITEK 2 Systems

Table 2: Optical density of clinical isolates for Quaternary ammonium disinfectant

Microorganisms	*Optical density (McF)*
S.*aureus* (MRSA)	1.03McF
K.*pneumoniae*	1.08McF
A.*baumannii*	1.02McF
P.*aeruginossa*	1.03McF
C.*tropicalis*	1.06McF

Now microbial suspension of positive control of 3ml of 5 different microorganism is ready.

3.0 ml of 0.4% Quaternary ammonium disinfectant in 5 different glass test tube by labeling the name of micro organisms we use in this test (S. *aureus* (MRSA), K. *pneumoniae*, A. *baumannii*, P.*aeruginossa*, C.*tropicalis*) is added.

1 ml of microbial load in each of test tube having pure form of 3 ml Quaternary ammonium disinfectant by using micropipette is added and mix well.

- After 5 minutes of incubation : 20 µl with micropipette of sample and streak it into 1 mackonkey agar labeled as Klebsiella pneumoniae 5 min plate and another 20µl with micropipette pass in 5 ml nutrient broth labeled as Klebsiella pneumoniae 5 min and also 20 µl of sample from microbial suspension Klebsiella pneumoniae and added into mackonkey agar plate with nutrient broth tube labeled as positive control of Klebsiella pneumonia.
- After 10 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Klebsiella pneumoniae 5 min plate and another 20µl with micropipette pass in 5 ml nutrient broth labeled as Klebsiella pneumoniae 10 min.
- After 15 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Klebsiella pneumoniae 5 min plate and another 20µl passed in 5 ml nutrient broth labeled as Klebsiella pneumoniae 15 min. Same protocol followed with another four organism with this 0.4% Alliance R82

3. By using Aldehyde free disinfectant

Concentration final volume to 10% as per usage in hospitals and laboratories 200µl of Aldehyde free disinfectant is added into a 20ml of distilled water and final volume of 10% Aldehyde free disinfectant is made .

By measuring the optical density of 5 different microorganisms suspension with help of VITEK DENSICHEK instrument is an accessory intended for use with the VITEK 2 Systems.

Table 3 : Optical density of clinical isolates for Aldehyde free disinfectant

Microorganisms	*Optical density (McF)*
S.*aureus* (MRSA)	1.01McF
K.*pneumoniae*	1.03McF
A.*baumannii*	1.05McF
P.*aeruginossa*	1.08McF
C.*tropicalis*	1.07McF

By adding 3ml of 10 Aldehyde free disinfectant in 5 different glass test tube by labeling the name of micro organisms we use in this test (S.*aureus* (MRSA), K. *pneumoniae*, A.*baumannii*, P.*aeruginossa*, C.*tropicalis*)

Add 1 ml of microbial load in each of test tube having pure form of 3 ml Aldehyde free disinfectant by using micropipette and mix well.

After 5 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Acinetobacter *baumannii* 5 min plate and another 20µl with micropipette pass in 5 ml nutrient broth labeled as Acinetobacter *baumannii* 5 min and also 20 µl of sample from microbial suspension Acinetobacter *baumannii* and added into mackonkey agar plate with nutrient broth tube labeled as positive control of Acinetobacter *baumannii.*

After 10 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Acinetobacter *baumannii* 5 min plate and another 20µl with micropipette and passed in 5 ml nutrient broth labeled as Acinetobacter *baumannii* 10 min.

After 15 minutes of incubation : 20 µl of sample and streaked it onto 1 mackonkey agar labeled as Acinetobacter *baumannii* 5 min plate and another 20µl with micropipette passed into 5 ml nutrient broth labeled as Acinetobacter *baumanni*i 15 min. *Same protocol followed with another four organism with this 10% Aldehyde free disinfectant.*

3 Results and Discussion:

Table 4: Summary of results

1. By using **Chlorine releasing disinfectant**	5 min incubation	10 min incubation	15 min incubation	Positive control
Culture plates		-	-	
Staphylococcus *aureus*	-			+
Acinetobacter *baumannii*	-	-	-	+
Pseudomonas *aeruginossa*	-	-	-	+
Klebsiella *pneumoniae*	-	-	-	+
Candida *tropicalis*	-	-	-	+
Broth culture				
Staphylococcus *aureus*	-	-	-	+
Acinetobacter *baumannii*	-	-	-	+
Pseudomonas *aeruginossa*	-	-	-	+
Klebsiella *pneumoniae*	-	-	-	+
Candida *tropicalis*	-	-	-	+
2. By using **Quaternary ammonium disinfectant**	5 min incubation	10 min incubation	15 min incubation	Positive control
Culture plates		-	-	
Staphylococcus aureus	-			+
Acinetobacter baumannii	-	-	-	+
Pseudomonas aeruginossa	-	-	-	+
Klebsiella pneumoniae	-	-	-	+
Candida tropicalis	-	-	-	+
Broth culture		-		+
Staphylococcus aureus	-		-	
Acinetobacter baumannii	-	-	-	+
Pseudomonas aeruginossa	-	-	-	+
Klebsiella pneumoniae	-	-	-	+
Candida tropicalis	-	-	-	+

3. By using **Aldehyde free disinfectant**	5 min incubation	10 min incubation	15 min incubation	Positive control
Culture plates				
Staphylococcus aureus	-	-	-	+
Acinetobacter baumannii	-	-	-	+
Pseudomonas aeruginossa	-	-	-	+
Klebsiella pneumoniae	-	-	-	+
Candida tropicalis	-	-	-	+
Broth culture				
Staphylococcus aureus	-	-	-	+
Acinetobacter baumannii	-	-	-	+
Pseudomonas aeruginossa	-	-	-	+
Klebsiella pneumoniae	-	-	-	+
Candida tropicalis	-	-	-	+

By using Chlorine releasing disinfectant -

Staphylococcus *aureus* - After 48 hours of incubation

Positive control | Culture after 5 min | Culture after 10min | Culture after 15min

Fig 1 :In figure culture shows no growth of Staphylococcus *aureus* in , 5min, 10min and 15 min but culture shows growth of Staphylococcus *aureus* in positive control of Staphylococcus *aureus* after 48 hours of incubation.

Fig 2: Figure shows no turbidity in negative control of Chlorine releasing disinfectant, 5min, 10min and 15 min but turbidity is seen in positive control of *Staphylococcus aureus* after 48 hours of incubation.

Klebsiella *pneumoniae* – After 48 hours of incubation

Positive Control	Culture after 5 min	Culture after 10min	Culture after 15min

Fig 3: In figure culture shows no growth of Klebsiella *pneumoniae* in 5min, 10min and 15 min but culture shows growth of Klebsiella *pneumonia* in positive control of Klebsiella *pneumonia* after 48 hours of incubation in positive control.

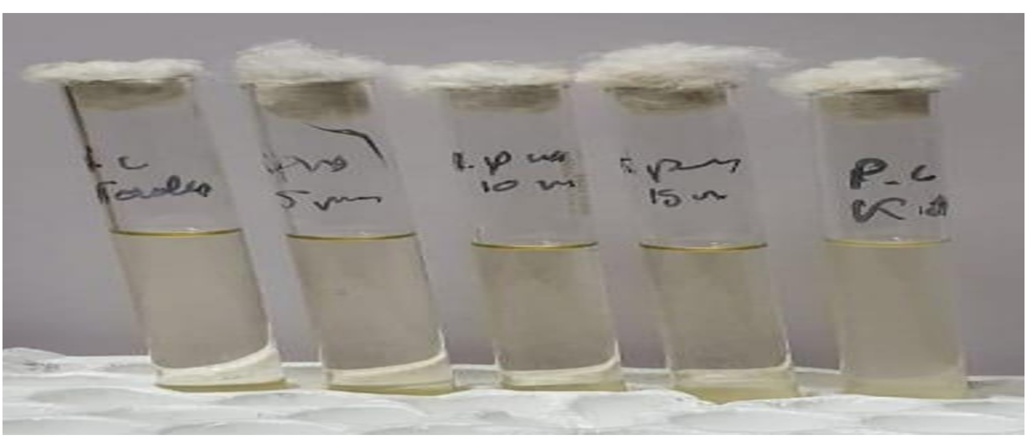

Fig 4.: Figure shows no turbidity in negative control of Chlorine releasing disinfectant, 5min, 10min and 15 min but turbidity is seen in positive control of Klebsiella *pneumoniae* after 48 hours of incubation.

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Acinetobacter *baumannii* - After 48 hours of incubation.

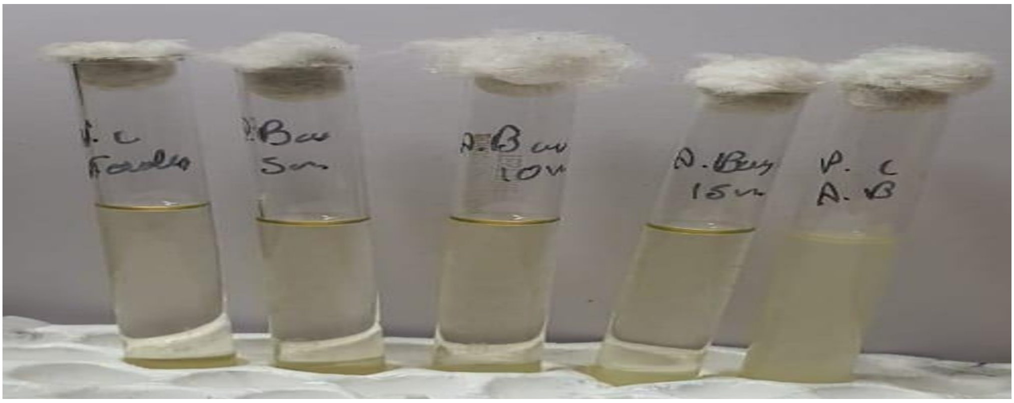


Fig 5: Figure shows no turbidity in negative control of Chorine releasing disinfectant , 5min, 10min and 15 min but turbidity is seen in positive control of Acinetobacter *baumannii* after 48 hours of incubation.

Candida *tropicalis* - After 48 hours of incubation

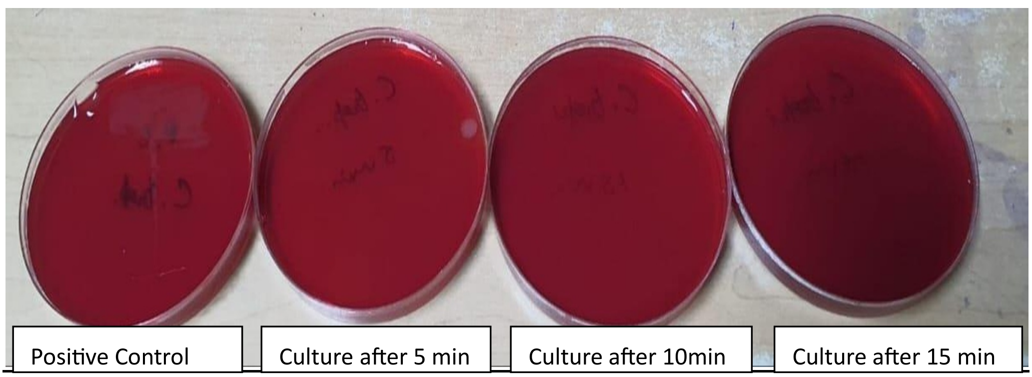
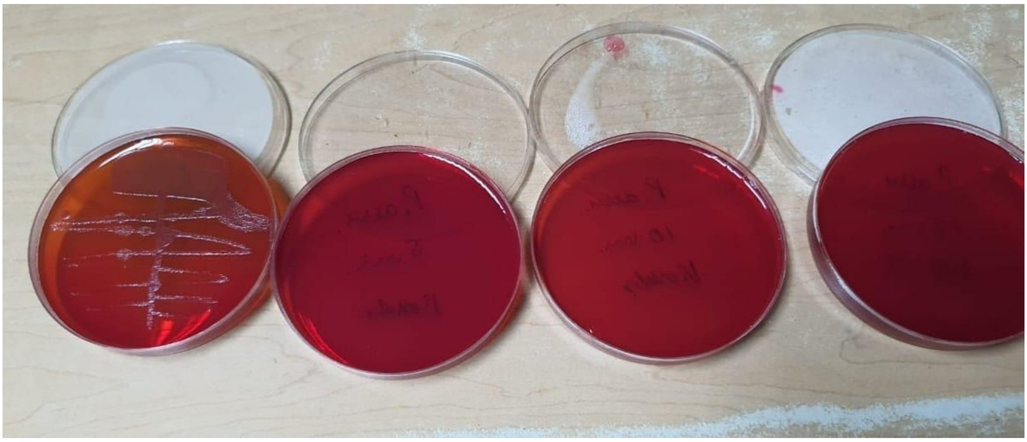


Fig 6: In figure culture shows no growth of Candida *tropicalis* in 5min, 10min and 15 min but culture shows growth of Candida *tropicalis* in positive control of Candida *tropicalis* after 48 hours of incubation in positive control.

Fig 7: Figure shows no turbidity in negative control of Chorine releasing disinfectant , 5min, 10min and 15 min but turbidity is seen in positive control of Candida *tropicalis* after 48 hours of incubation .

Pseudomonas *aeruginossa* - After 48 hours of incubation

Fig 8 : Figure shows no turbidity in negative control of Chorine releasing disinfectant, 5min, 10min and 15 min but turbidity seen in positive control of Pseudomonas *aeruginossa* .



Positive control	Culture after 5 min	Culture after 10 min	Culture after 15 min

Fig 9 : In figure culture shows no growth of Pseudomonas *aeruginossa* in 5min, 10 min and 15 min but culture shows growth of Pseudomonas *aeruginossa* in positive control of Pseudomonas *aeruginossa* after 48 hours of incubation.

By using Aldehyde free disinfectant Staphylococcus *aureus* –

After 48 hours of incubation.

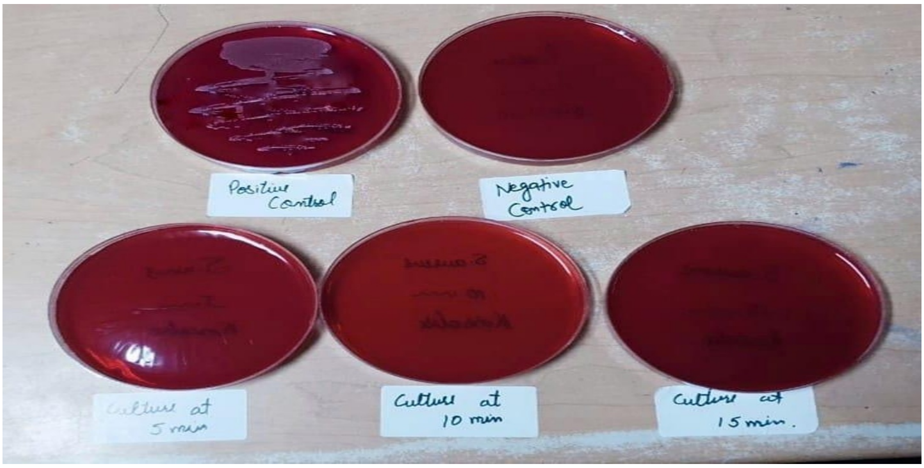


Fig 10: In figure culture shows no growth of Staphylococcus *aureus* in negative control of Aldehyde free disinfectant, 5min, 10min and 15 min but culture shows growth of Staphylococcus *aureus* in positive control of Staphylococcus *aureus* after 48 hours of incubation.

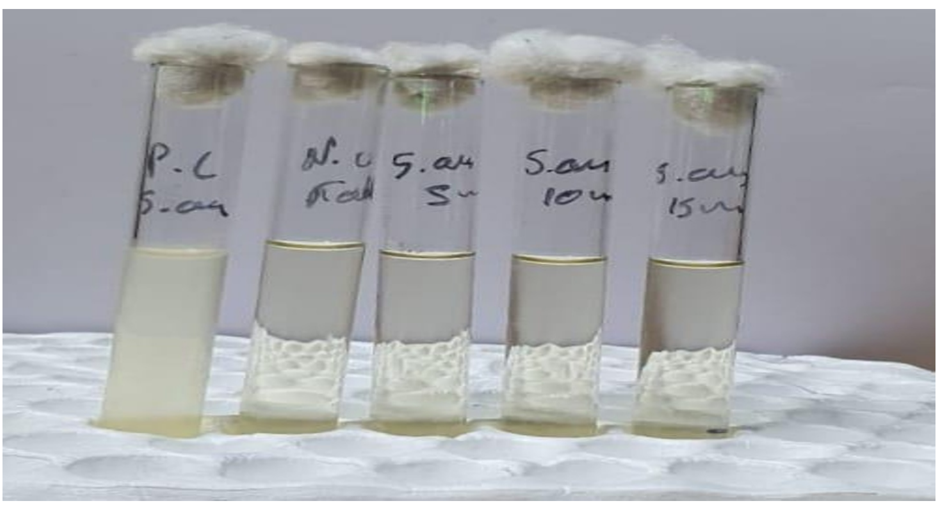


Fig 11: Figure shows no turbidity in negative control of Aldehyde free disinfectant, 5min, 10min and 15 min but turbidity is seen in positive control of Staphylococcus *aureus* after 48 hours of incubation.

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Klebsiella *pneumoniae* - After 48 hours of incubation

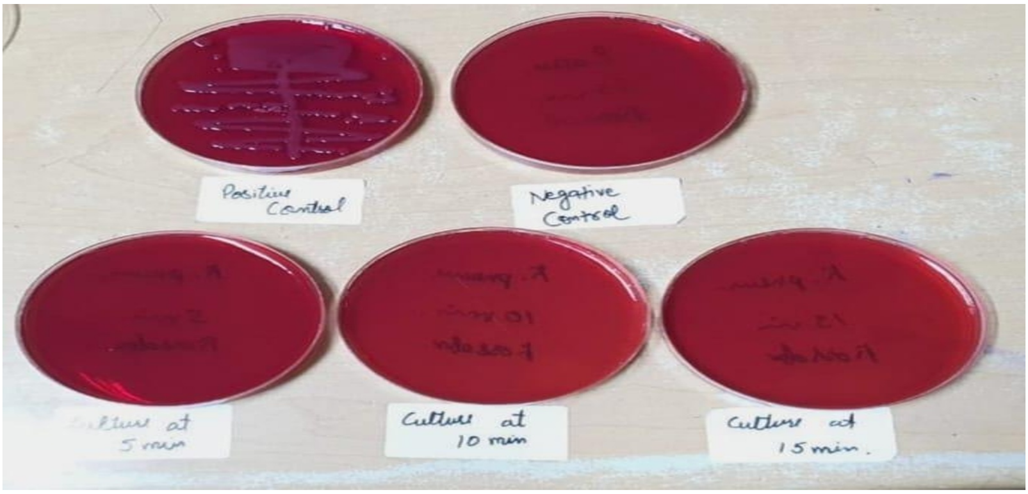

Fig 12 : In figure culture shows no growth of Klebsiella *pneumonia* in negative control , 5min, 10min and 15 min but culture shows growth of Klebsiella *pneumonia* in positive control of Klebsiella *pneumoniae* after 48 hours of incubation.

Fig 13: Figure shows no turbidity in negative control of Aldehyde free disinfectant, 5min, 10min and 15 min but turbidity is seen in positive control of Klebsiella *pneumoniae* after 48 hours of incubation.

3 Discussion

Different countries have regulatory bodies that establish standards and guidelines for disinfectant efficacy testing. These standards define the testing methods, organisms used, and performance criteria that a disinfectant must meet to be considered effective.

In Kelsey Sykes test - The disinfectant is challenged by three successive additions of a bacterial suspension during the course of the test.

All these three disinfectants (chlorine releasing disinfectant, Aldehyde free disinfectant, Quaternary ammonium compound) used in my project which commonly used in hospitals and laboratories.

These are very effective in inhibiting the growth of the selected bacterial strains at 5 min, 10min and 15 min of incubation.

As firstly test was performed on chlorine releasing disinfectant which show very high tendency of inhibiting the growth at 5 minutes of incubation, 10 minutes of incubation and 15 minutes of incubation. After 48 hours of incubation there is not any bacterial growth on the on the cultures of mackonkey agar and turbidity in nutrient broth. As already discussed above that the recommendation time of contact of chlorine releasing disinfectant is 10 min. So in this experiment disinfectant was also cultured at 5 min time of contact after 48 hours of incubation which means this disinfectant is also effective at 5 minutes of time of contact.

Secondly test was performed on aldehyde free disinfectant which show very high tendency of inhibiting the growth at 5 minutes of incubation, 10 minutes of incubation and 15 minutes of incubation. After 48 hours of incubation there is not any bacterial growth on the on the cultures of MacConkey agar and turbidity in nutrient broth. As already discussed above that the recommendation time of contact of aldehyde free disinfectant is 10 min. So, in this experiment disinfectant was also cultured at 5 min time of contact after 48 hours of incubation which means this disinfectant is also effective at 5 minutes of time of contact.

At last test was performed on quaternary ammonium disinfectant which show very high tendency of inhibiting the growth at 5 minutes of incubation, 10 minutes of incubation and 15 minutes of incubation. After 48 hours of incubation there is not any bacterial growth on the on the cultures of MacConkey agar and turbidity in nutrient broth. As already discussed above that the recommendation time of contact of on quaternary ammonium disinfectant is 10 min .

So in this experiment disinfectant was also cultured at 5 min time of contact after 48 hours of incubation which means this disinfectant is also effective at 5 minutes of time of contact.

As It did not show any culture growth in all culture and no turbidity in nutrient broth the rate of the inhibition of these products was also higher than the recommendation time of contact. Therefore, the usage of these disinfectants (Chlorine releasing disinfectant, Quaternary ammonium compound, Aldehyde free disinfectant) are showing better effectiveness to ensure having high quality disinfectants for hospitals and the community.

So, in this project Kelsey Sykes method is used to perform which is the triple-challenge test is used to find the optimal disinfectant concentrations for both unclean and clean environments. Throughout the process, the disinfectant is added to a bacterial suspension three times in succession.

It takes minimum 15-20 minutes to complete the test which is time saving, easy to perform at any clinical setup, no more equipments and instruments is needed but some cons of this test are that pH may affect the action of disinfectant by entered into a microbial suspension of different bacterial isolates, the contact between aqueous solution of disinfectant is increased if they have surfactant properties [49].

Disinfectants have specified contact times and concentrations recommended by manufacturers. These parameters are crucial as they influence the efficacy of the product. Insufficient contact time or inadequate concentration may reduce the disinfectant's effectiveness.

There are some factors which generally make a significant impact on efficacy of disinfectants

1. Contact Time: Contact time refers to the duration that a disinfectant needs to remain in contact with the surface or area being treated to effectively kill or inactivate microorganisms. Different disinfectants have specified contact times recommended by manufacturers, and it is crucial to follow these instructions. Inadequate contact time can result in incomplete disinfection, as the microorganisms may not be exposed to the disinfectant for a sufficient period to be effectively eliminated.

2. Concentration: Disinfectants are formulated at specific concentrations, and using the correct concentration is vital for achieving effective disinfection. The concentration of a disinfectant determines its potency and ability to kill or inactivate microorganisms. Using a higher concentration than recommended may not necessarily improve efficacy and can lead to safety concerns, such as increased toxicity or corrosiveness. Conversely, using a lower concentration may not effectively eliminate microorganisms. Adhering to the recommended concentration ensures optimal disinfection results.

3. Real-World Application Conditions: Disinfection in real-world settings is influenced by various environmental conditions that can impact efficacy. Some of these conditions include:

Organic Matter: Presence of organic matter, such as blood, saliva, or feces, on surfaces can reduce the effectiveness of disinfectants. Organic matter can act as a physical barrier and interfere with the contact between the disinfectant and microorganisms. Thorough cleaning and removal of organic matter before disinfection are essential for optimal efficacy.

Temperature: Disinfectants may exhibit different efficacy at various temperatures. Some disinfectants work better at specific temperature ranges, and deviations from the recommended temperature may affect their effectiveness. It is important to consider temperature requirements and ensure appropriate conditions during disinfection.

4.Humidity: High humidity levels can impact the drying time of disinfectants, potentially affecting their efficacy. Additionally, certain microorganisms may be more resistant to disinfection in high humidity environments. Adapting disinfection practices to accommodate varying humidity conditions can help maintain efficacy.

Adhering to manufacturer instructions and recommended practices is crucial for achieving optimal disinfection results. Manufacturers provide specific guidelines regarding contact time, concentration, and application procedures based on rigorous testing and scientific data. Following these instructions ensures that the disinfectant is used correctly and maximizes its efficacy[50].

By considering these factors and following recommended practices, disinfectants can be applied effectively, leading to successful microbial control and improved infection prevention in various settings, such as healthcare facilities, households, and public spaces.

4 Conclusion

There are many types of methods to check efficacy of different types of disinfectants which are used in studies to demonstrate the efficacy of disinfectants used on surfaces in manufacturing areas, laboratories and other facility areas are effective in inactivation or removal of microorganisms, such as bacteria, fungi (yeast and molds), bacterial spores, viruses and mycoplasma. Disinfectant studies can support cleaning studies by showing that application of the disinfectant reduces or eliminates microorganisms, but they should not be considered a substitute for establishing that the cleaning agents and physical cleaning actions are acceptable. So in this project disinfectants shows very good response against different microorganisms and inhibit its growth within few minutes of time of contact . It means that these three disinfectants (Chlorine releasing disinfectant, Quaternary ammonium compound, Aldehyde free disinfectant) can be used anywhere in hospitals, laboratories and surgical area which can decontaminant the surfaces very easily.

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