AN *IN VITRO* STUDY TO EVALUATE THE ANTI-MICROBIAL EFFECT OF *NAGAKESARADI DHOOPANA YOGA* IN HOSPITAL ROOM

ABSTRACT

Introduction: Nosocomial diseases are the Hospital acquired disease which can be considered as the one of the main reason for contagious disease in hospital. Among the microorganisms which are responsible for nosocomial disease *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger* are the main strains. Hence maintaining the air hygiene is very essential in to prevent such spread. Formaldehyde fumigation is the standard procedure which is followed in hospital in order to fumigate OT as the part of sterilisation. But formaldehyde is a potent carcinogen and is also capable of causing various health hazards. Hence *Dhoopana* can be taken up as the measure to maintain the air hygiene without health effects. *Nagakesaradi Dhoopana Yoga* which explained in *Kriyakoumudi* in the context of *Jaladhi shudhikarana* is directly indicated in the *Vishavayu* and against microorganisms. **Materials and methods:** An in-vitro study was carried out with *Nagakesaradi Dhoopana Yoga*. Multiple swabs were collected from different parts of in-patient rooms, cultured and *Dhoopana* of the same rooms were done. Later swabs were collected from the same places and the results were compared. **Observations and Results:** The study shows that *Nagakesaradi Dhoopana Yoga* has very good action in preventing the microbial growth in hospital rooms. **Conclusion:** The *Nagakesaradi Dhoopana Yoga* has better anti-bacterial and anti- fungal activity.

Keywords: *Dhoopana, Nagakesaradi Dhoopana Yoga,* microorganisms Anti-bacterial activity, Anti-fungal activity

INTRODUCTION

Indoor Air Quality (IAQ) is the air quality within and around buildings and structures. IAQ is known to affect the health, comfort, and well-being of building occupants. Poor indoor air quality has been linked to sick building syndrome, reduced productivity, and impaired learning in schools. [1] However, these diseases can also be traced to microorganisms that grow in home heating and cooling systems and humidifiers. Children, elderly people, and people with breathing problems, allergies, and lung diseases are particularly susceptible to disease-causing biological agents in the indoor air. Tuberculosis, measles, Staphylococcus

infections, Legionella and influenza are known to be transmitted by air. [2] Among the most important bacteria known to occur in indoor air are *Mycobacterium tuberculosis, Staphylococcus aureus, and Streptococcus pneumoniae*. [1]

Nosocomial infections affect a substantial number of patients globally, leading to increased mortality and financial impact on healthcare systems. The most common route of transmission is through contact, whereby the organisms are transmitted by direct or indirect contact. Droplet transmission may occur when microorganisms are transmitted from the respiratory tract by large droplets (greater than 5 microns) and travel less than 3 feet. Airborne transmission involves the transmission of organisms from the respiratory tract by small droplets (less than 5 microns) that travel long distances. [3] Chemical fumigation is now being applied as a nosocomial infection control measure in the health care environment because of the difficulty in completely disinfecting rooms and equipment. Fumigation is being considered because gases and vapours can permeate areas that are not easily reachable. [4] *Nagakesaradi Dhoopana Yoga* is explained in *Kriyakoumudi*, Malayalam *Visha Chikitsa* textbook written by V.M. Kuttikrishna Menon in the context of *Jaladhi Sudhikarana. Nagakesara, Daruharidra, Ela, Twak, Kushta, Priyangu, Laksha, Ativisha, Musta, Nirgundi* are the drugs which is explained in this *Yoga*

OBJECTIVE

To evaluate the anti-microbial effect of *Dhoopana Karma* with *Nagakesaradi Dhoopana Yoga* in in-patient room

MATERIAL AND METHODS

Preparation and Standardization of *Nagakesaradi Dhoopana Yoga*

All the drugs were collected in equal quantity, authenticated and Choorna (Average Coarse Powder -4mm mesh size) were prepared as per the general method from G.M.P. certified S.D.M. Ayurveda Pharmacy, Kuthpady, Udupi, Karnataka, India. Ingredients of *Nagakesaradi Dhoopana Yoga* are tabulated in Table no.1.

All the Analytical tests including HPTLC were conducted from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.

Table no.1; Ingredients of *Nagakesaradi Dhoopana Yoga* [5- 7]

Sl. No.	Drugs	Botanical Name	Part Used
1.	***Nagakesara***	*Mesua ferrea* Linn.	Stamens
2.	***Daruharidra***	*Berberis aristata* DC.	Root
3.	***Ela***	*Elettaria cardamomoum* (Linn.) Maton	Fruits and seeds
4.	***Twak***	*Cinnamomum zeylanicum* Blume.	Stem Bark
5.	***Kushta***	*Saussurea lappa* C.B. Clarke	Root
6.	***Priyangu***	*Callicarpa macrophylla* Vahl.	Seeds
7.	***Laksha***	*Laccifer lacca* (Kerr).	Resin
8.	***Ativisha***	*Aconitum heterophyllum* Wall. ex Royle	Tuberous root
9.	***Musta***	*Cyperus rotundus* Linn.	Tubers
10.	***Nirgundi***	*Vitex negundo* Linn.	Leaves

Experimental Source

Study was conducted in two non-sterile In-Patient rooms of size 168 sq. ft. (14'*12') of S.D.M. Ayurveda Hospital, Kuthpady, Udupi

Materials used for the study:

1. Two non –sterile In-Patient rooms of size 168 sq. ft. (14'*12')

2. Two *Mrit sharavas*, was obtained from local market

3. *Nagakesaradi Dhoopana Yoga*

4. Materials required for collecting swabs, counting and assessing microbial load were taken from S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi.

Selection of In-Patient room for *Dhoopana:*

- Two non- sterile In-Patient room of size 168 sq. ft. (14'*12') was selected from the Hospital.
- Room was named as Room A (Special room-325) and Room B (Special room- 310)
- Room was kept closed for 24 hours after the discharge of the patient without any disinfection procedures.
- Any experiment was conducted after 24 hours.

Preparation of microorganisms:

Both Casein Soya bean Digest Agar Medium (CSDAM) and Sabaud's Dextrose Agar medium were prepared for bacteria and fungus respectively.

Preparation of swab:

- Both hands and the rod were sterilized priorly.
- Sterile absorbent cotton was taken and rolled over the rod.
- Prepared swabs were autoclaved at 121° C for 15 mins.

Preparation of *Dhoopa* in *Sharava:*

- Charcoal was ignited with the help of Ghee and made in to red hot
- 2 *Mrit Sharavas* was taken and added with sufficient quantity of charcoal
- Later 100gms of *Nagakesaradi Dhoopana Yoga* was divided and added to the igniting charcoal in 2 *Sharavas.*

Experiment procedure:

- Swabs were collected from 7 sites of selected room before *Dhoopana*
- Sites from where swabs are collected:-
    a) Wall
    b) Curtain

c) Chair
d) Switch
e) Bed
f) Bathroom door handle
g) Cupboard door handle

- These sites were marked with marker.
- 2 *Mrita Sharavas* with fumes were kept inside the room in center.
- All ventilators, windows, doors etc. were closed tightly in order to avoid the spillage of fumes out of the room.
- *Dhoopana* was done for 45 mins.
- After 45 mins both *Sharavas* were taken out and room was kept closed for further 2 hours by maintaining the fumes inside the room.
- After 2 hours room was opened and fumes was allowed to escape.
- Later multiple swabs were collected from the previously marked sites.
- Swabs were sent to the Research lab for further inoculation and microbial load analysis.

Inoculation of microorganism:

- The collected swabs were kept inside the laminar air flow chamber.
- Swabs were dipped in 10 ml saline and properly mixed and kept.
- Petri dish was taken and added with suitable media as per the strain, later added with 1 ml of solution from sample and mixed uniformly for proper spreading of strains.
- Kept aside and allowed to get solidify.

Microbial load Analysis:

- After the procedure, Petri dish of bacteria was kept incubation in Incubator for 24 hours in 37° C and fungus was kept incubation in BOD (Biological Oxygen Demand) for 4 days in 25° C.
- After incubation microbial load counting was done with the help of Digital Colony Counter.
- Comparison of microbial load was done with before and after *Dhoopana*.

Figure no. 1; Procedure foolowed for the experiment

Figure no.1a; Collecting swabs before *Dhoopana*

Figure no.1b; *Nagakesaradi Dhoopana Yoga* was weighed

Figure no.1c ; *Sharavas* was kept inside the Room

Figure no.1d; Room was completely closed

Figure no.1e ; Swabs were collected after *Dhoopana*

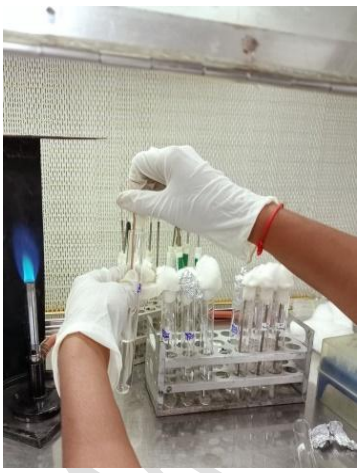


Figure no.1f; Dipping swabs in saline

Figure no.1g; Pouring medium in to the petridish

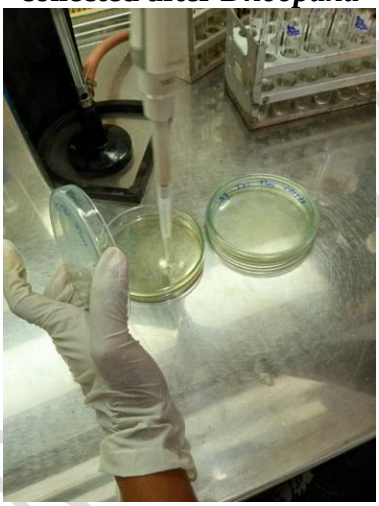


Figure no.1h; Adding the sample to the medium

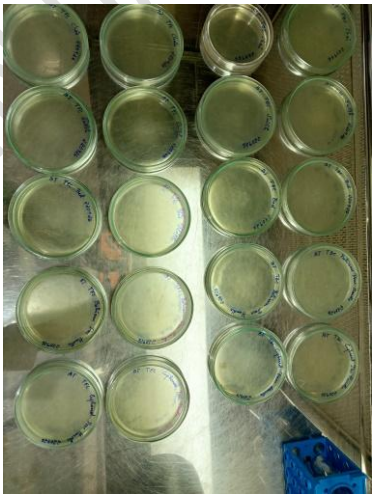


Figure no.1i ; All petridish were labeled (BT and AT) and kept for solidifying

OBSEVATIONS

Room A (Special room-325)

Table no. 1a; Total Bacterial Count of swabs - Before treatment & after treatment in Room A

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	03	0
2	Curtain	01	0
3	Chair	10	02
4	Switch	12	0

5	Bed	19	0
6	Bathroom door handle	10	03
7	Cupboard door handle	03	0

Table no. 1b; Total Fungal Count of swabs- Before and after treatment in Room A

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	02	0
2	Curtain	88	45
3	Chair	02	01
4	Switch	0	0
5	Bed	06	05
6	Bathroom door handle	0	0
7	Cupboard door handle	06	01

Observations

- **Wall:** Number of bacterial colony was reduced from 3 to nil and fungal colony was reduced from 2 to nil, showing remarkable reduction after *Dhoopana*.
- **Curtain:** Number of bacterial colony was reduced from 1 to nil and fungal colony was reduced from 88 to 45 after *Dhoopana*.

- **Chair:** Number of bacterial colony was reduced from 10 to 2 and fungal colony was reduced from 2 to 1, showing remarkable reduction after *Dhoopana*.
- **Switch:** Number of bacterial colony was reduced from 12 to nil, showing remarkable reduction after *Dhoopana*. No fungal colonies were found before and after treatment.
- **Bed:** Number of bacterial colony was reduced from 19 to nil, showing remarkable reduction and fungal colony was reduced from 6 to 5, after *Dhoopana*.
- **Bathroom door handle:** Number of bacterial colony was reduced from 10 to 3, showing remarkable reduction after *Dhoopana*. No fungal colonies were found
- **Cupboard door handle:** Number of bacterial colony was reduced from 3 to nil and fungal colony was reduced from 6 to 1, showing remarkable reduction after *Dhoopana*.

Room B (Special room-310)

Table no. 2a; Total Bacterial Count of swabs - Before treatment & after treatment in Room B

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT
1	Wall	0	0
2	Curtain	3	0
3	Chair	28	01
4	Switch	01	0
5	Bed	21	03
6	Bathroom door handle	04	01
7	Cupboard door handle	0	0

Table no. 2b; Total Fungal Count of swabs- Before and after treatment in Room B

Sl. No.	Name	Number of Colonies (NOC)	
		BT	AT

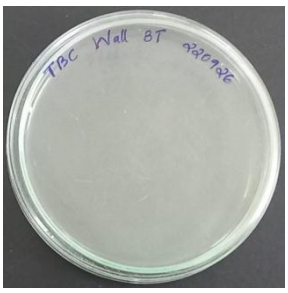
1	Wall	0	0
2	Curtain	54	15
3	Chair	09	01
4	Switch	01	0
5	Bed	04	07
6	Bathroom door handle	01	0
7	Cupboard door handle	01	0

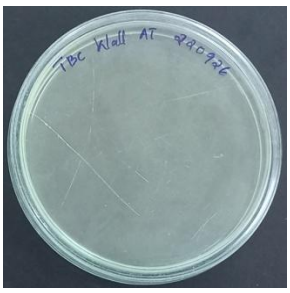
Observation

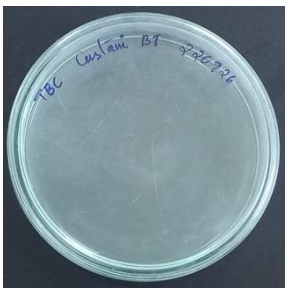
- **Wall:** There were no bacterial and fungal colonies found before and after treatment.
- **Curtain:** Number of bacterial colony was reduced from 3 to nil and fungal colony was reduced from 54 to 15, showing remarkable reduction after *Dhoopana*.
- **Chair:** Number of bacterial colony was reduced from 28 to 1 and fungal colony was reduced from 9 to 1, showing remarkable reduction after *Dhoopana*.
- **Switch:** Number of bacterial colony was reduced from 1 to 0 and fungal colony was reduced from 1 to 0.
- **Bed:** Number of bacterial colony was reduced from 21 to 3, and fungal colony showed slight increase from 4 to7.
- **Bathroom door handle:** Number of bacterial colony was reduced from 4 to 1, and fungal colony was reduced from 1 to 0, showing remarkable reduction after *Dhoopana*.
- **Cupboard door handle:** No bacterial colonies were found and fungal colony was reduced from 1 to 0.

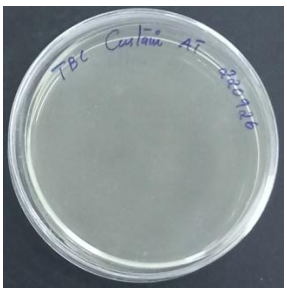
Room A

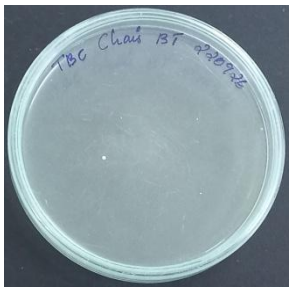
Figure no.2; Total Bacterial Count of swabs - Before treatment & after treatment


Wall-BT

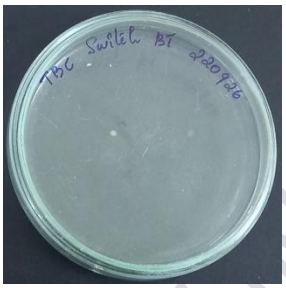

Wall-AT

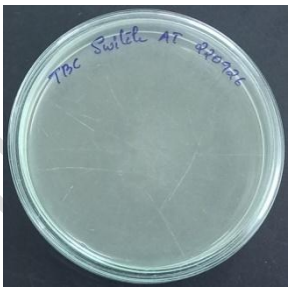

Curtain-BT

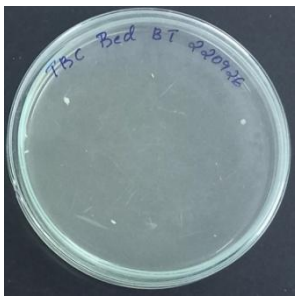

Curtain-AT

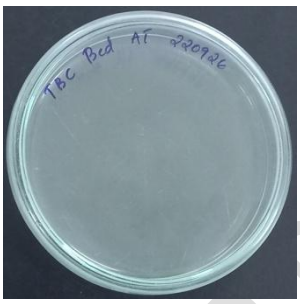

Chair-BT


Chair-AT

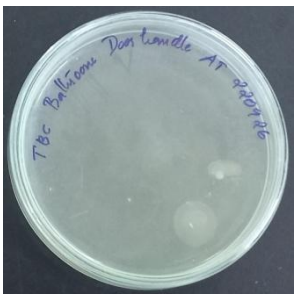

Switch-BT

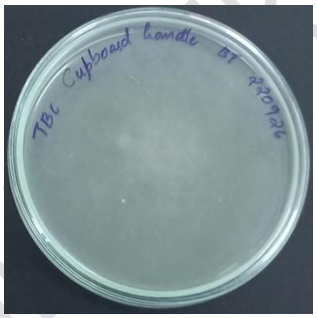

Switch-AT


Bed-BT


Bed-AT


Bathroom door handle-BT


Bathroom door handle-AT


Cupboard door handle-BT

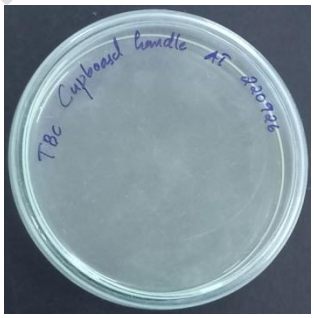
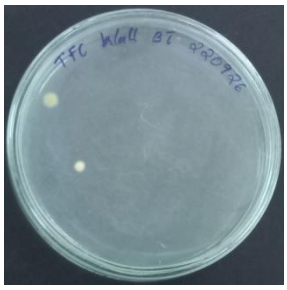
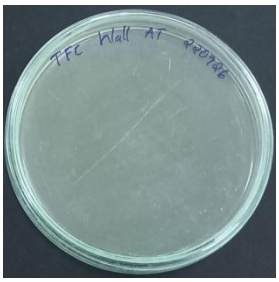
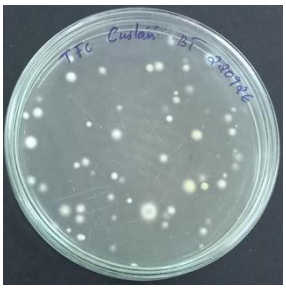
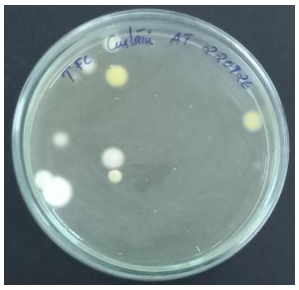

Cupboard door handle-AT

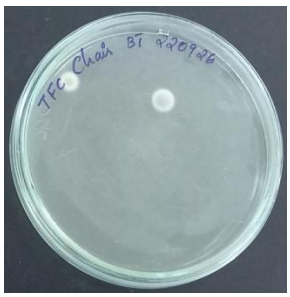
Figure no.3; Total Fungal Count of swabs - Before treatment & after treatment


Wall-BT


Wall-AT

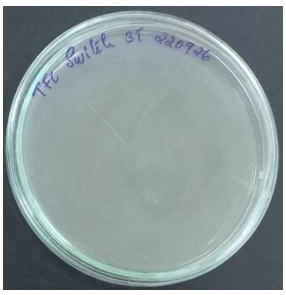

Curtain-BT


Curtain-AT

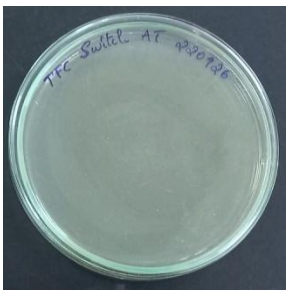


Chair-BT

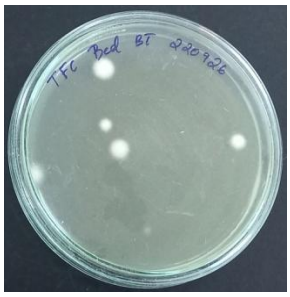
Chair-AT



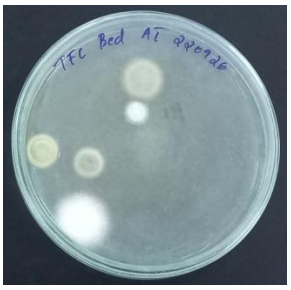
Switch-BT



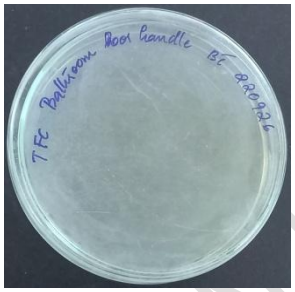
Switch-AT



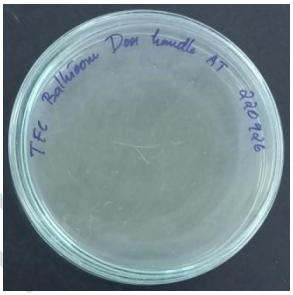
Bed-BT



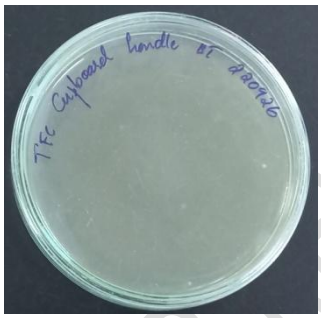
Bed-AT



Bathroom door handle-BT



Bathroom door handle-AT



Cupboard door handle-BT

Cupboard door handle-AT

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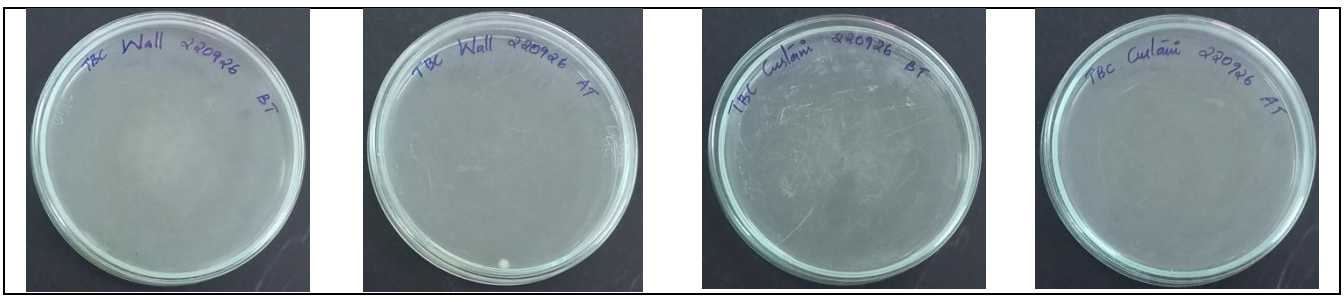
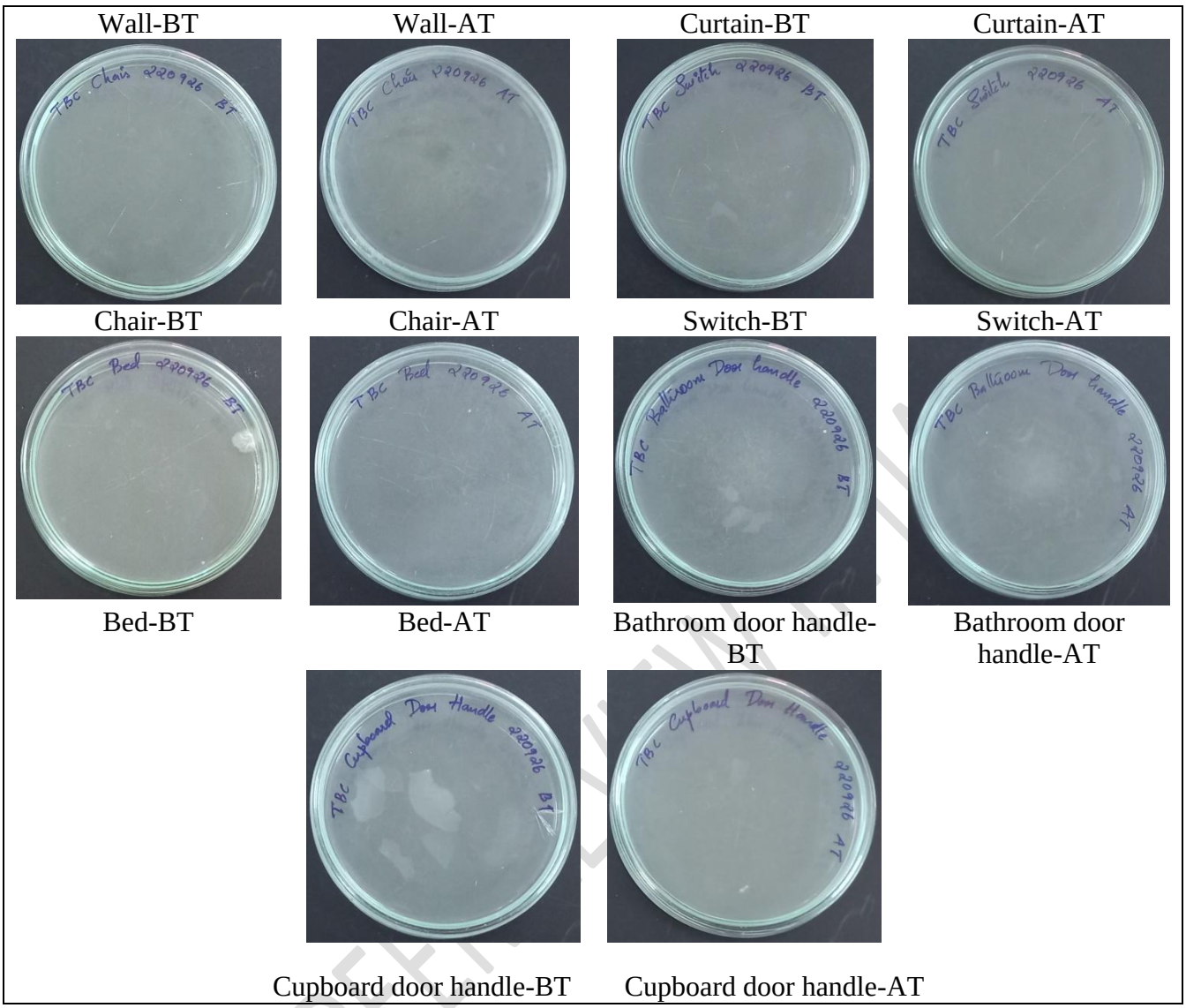
205 **Room B**

206 **Figure no.4; Total Bacterial Count of swabs - Before treatment & after treatment**





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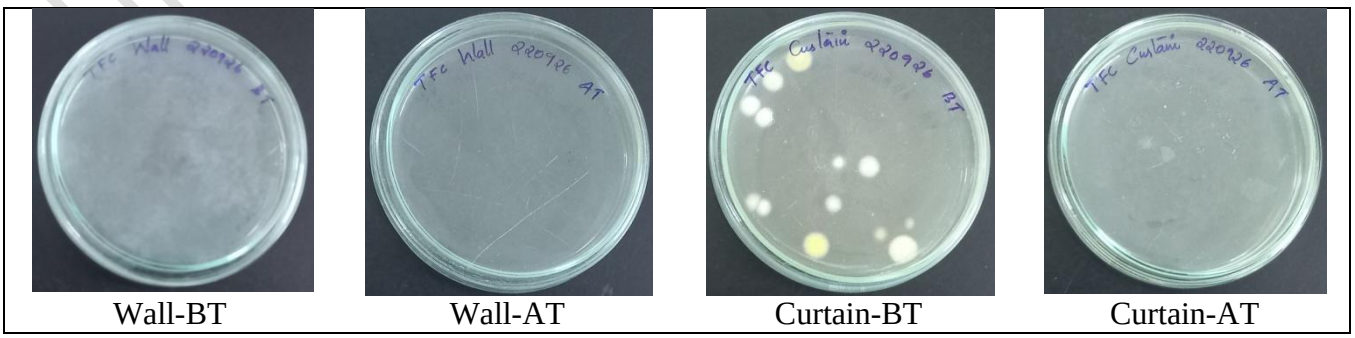
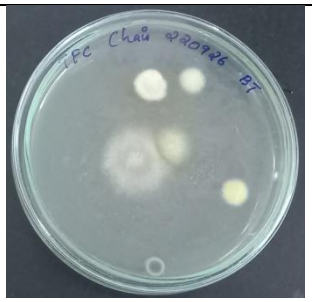
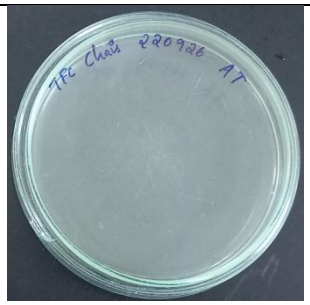
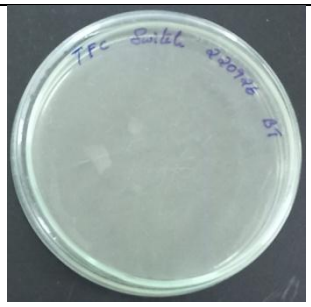
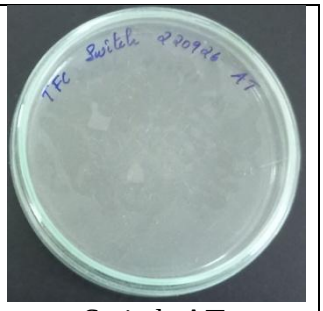
210 **Figure no.5; Total Fungal Count of swabs- Before and after treatment**




Chair-BT

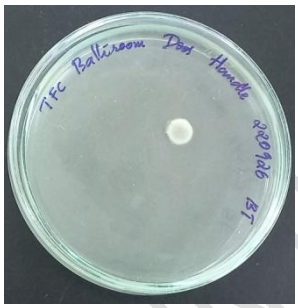

Chair-AT


Switch-BT

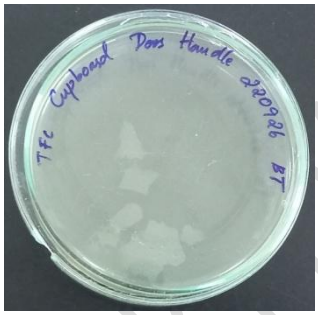

Switch-AT

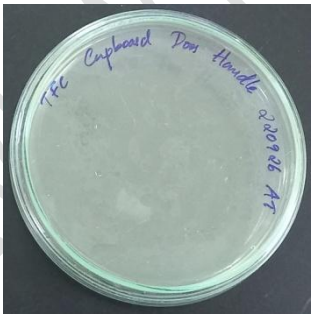

Bed-BT


Bed-AT


Bathroom door handle-BT


Bathroom door handle-AT


Cupboard door handle-BT


Cupboard door handle-AT

RESULTS AND DISCUSSION

Room A (Special Room- 325)

Dhoopana with 100 gms of *Nagakesaradi Dhoopana Yoga* showed better results as it reduced the microbial colony formation. It has shown both anti- bacterial and anti- fungal activity.

Room B (Special Room- 310)

Dhoopana with 100 gms of *Nagakesaradi Dhoopana Yoga* showed better results as it reduced the microbial colony formation. It has shown both anti- bacterial and anti- fungal activity. But a small amount fungal colony was found in Bed after Dhoopana, which may be due the inadequate exposure of fumes over that particular site or may be due to the activity of

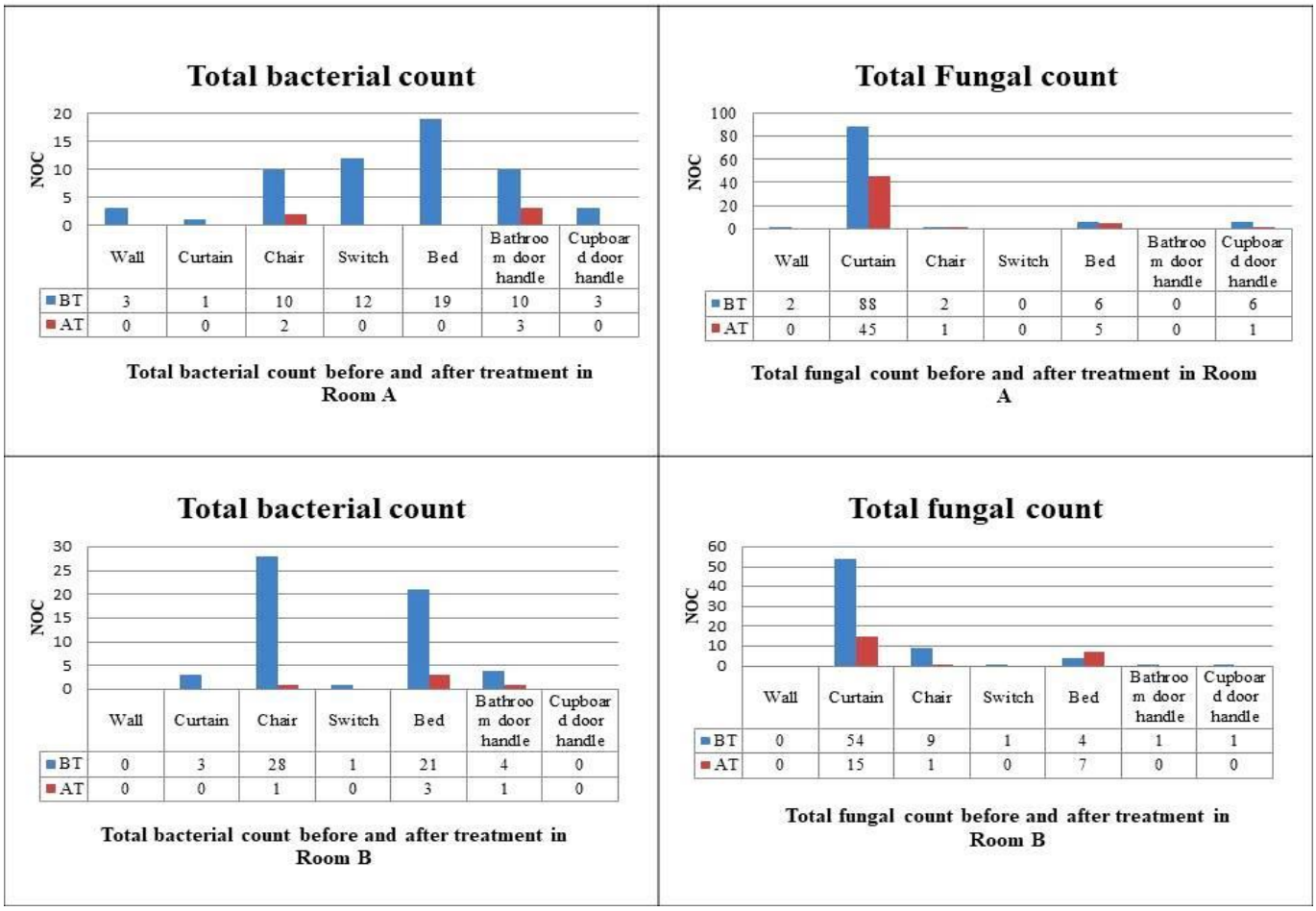
any particular strain of microorganism. Isolation of that particular strain and further study on that may help to know the reason for the growth. The results are depicted in Figure no:5

The presence Alkaloids, Carbohydrates, Tannins, Saponins, Coumarins and Carboxylic acid in Phytochemical analysis and Eugenol in HPTLC indicates the anti-microbial activity of the *Yoga* as these chemical constituents are proved for their anti-microbial activities. [8]

. **Figure no.6; Microbial analysis in Room A and B**

CONCLUSION

Maintaining indoor air quality is equally importance as outdoor air quality, as it can help to prevent various contagious diseases. The Experimental study in Hospital rooms shows that *Nagakesaradi Dhoopana Yoga* has good anti-bacterial and anti-fungal activity. *Dhoopana* of



100gms of *Nagakesaradi Dhoopana Yoga* for 45 mins, keeping room closed for further 2

hours shows better results in reduction of bacterial and fungal growth. *Dhoopana* in hospital room can help to prevent the nosocomial diseases. Hence study concludes that *Nagakesaradi Dhoopana Yoga* shows better results on microorganisms in indoor environment and can be considered as an effective disinfectant.

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