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Comparative Analysis of Hospital Microflora and Study of Disinfectant Activity

Bincy Mary Biju[1], Bhavyan Pandya[1], Rahul Yadav[1], Ruchita Rane[2]

[1,2]University of Mumbai
[1,2] S. S & L. S Patkar College of Arts & Science and V. P. Varde College of Commerce & Economics, Department of Biotechnology, Mumbai, India

Abstract: **Nosocomial infections are those which are acquired or developed during hospital stay or visit. It is widely accepted that the surface contamination play role in transmission of infection in the domestic and community setting. Usually hospital fomite such as door knobs, syringes, patient's bed, chair, desk and many such are possible objects which can acquire infection. The most common nosocomial infections involved are urinary tract infection, respiratory tract infection, gastrointestinal tract infection etc. The main aim of current study is to check level of fomite micro flora of different hospitals and steps that should be taken to control the transmission of disease from fomite. A survey of two government and two private hospitals was done. Microbial load of air by air sedimentation method & fomite was studied. The swab samples were collected from fomite surface such as Clinical Waste Decomposition Room, Water Reservoir, Dressing Tray, and General Ward. Microscopic & macroscopic characteristics were determined and isolates were identified using biochemical tests and Bergey's manual of systematic bacteriology. Further disinfectant activity of Dettol and Phenyl derivative were studied on common organism observed in both private and government hospital by Agar well diffusion method.**

Keywords: **Nosocomial infection, Fomite, Disinfectant activity.**

I. INTRODUCTION

Medical microbiology is the study of the interactions between animals and microorganisms such as bacteria, viruses, fungi, and parasites. Microorganisms play a critical role in human survival. The normal commensal population of microbes participates in the metabolism of food products, provides essential growth factors, protects against infections with highly virulent microorganisms, and stimulates the immune response. In the absence of these organisms life would be impossible.

Changes in health can disrupt the balance that is maintained among the organisms for example, hospitalization can lead to the replacement of normally a virulent organisms in the oropharynx with Gram-negative rods (e.g., *Klebsiella, Pseudomonas*) that can invade the lungs and cause pneumonia. Similarly, the indigenous bacteria present in the intestines restrict the growth of *Clostridium difficile* in the gastrointestinal tract. In the presence of antibiotics this indigenous flora is eliminated, and *C. difficile* is able to proliferate and produce diarrheal disease and colitis [1].

Hospital acquired infection (HAI) commonly called as nosocomial infection is the fourth most common cause of mortality after cardiovascular system disease, cancer and stroke. Infections of surgical wounds, Urinary Tract Infections and lower respiratory tract infections are most frequent nosocomial infections [2]. Fomites are porous or nonporous surfaces or objects which when contaminated with pathogenic microorganisms can serve as a transmission vehicle for that microorganisms [3]. The transfer of infectious microorganisms occurs between hands to fomite, fomite to hand, hand to hand, hand to fomite to hand or fomite to hand to fomite [4].

There is a significant role of fomites in transmission of various diseases; however the extent of their contribution in overall rate of nosocomial infections is unknown [5]. There is increase in nosocomial infections caused by Antibiotic resistant pathogens. Resistance is usually caused due to reasons such as antibiotic use and infection control practices &

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transmission of resistant strains by cross colonization of patients within hospitals [6]. Current investigation focuses on to isolate and identify hospital fomite micro flora and to assess effect of disinfectants on microorganisms commonly observed in hospital fomites.

II. MATERIALS AND METHODOLOGY

A. Collection of sample:

Samples were collected from Clinical waste decomposition room, Water reservoir, Dressing tray and General ward using sterile cotton swab. Air flora from patients' washroom was collected using Gravity Sedimentation method with exposure time of 1 minute and 5 minute [7].

B. Isolation of microorganisms:

The collected samples were isolated on sterile Nutrient agar medium, sterile Sabourauds' agar medium and sterile Mac Conkey's agar medium. Nutrient agar and Mac Conkey's agar plates were incubated at 37^0C for 24 hrs whereas Sabourauds' agar plates at room temperature for 48 hrs. The number of colonies obtained on each plate were counted and sedimentation rate was calculated [8].

C. Purification of culture:

To obtain pure culture, each isolate was sub cultured on sterile Luria Bertani and Sabourauds' agar slants and preserved for further work in refrigerator.

D. Macroscopic & morphological characterization of isolates:

Macroscopic and morphological characteristics of bacterial isolates were determined by standard procedure of Gram staining. Morphology of fungi was studied by wet mount staining.

E. Biochemical characterization of bacterial isolates:

Identification of bacterial isolates was done by performing biochemical tests [9] and comparing obtained results with Bergey's Manual of systematic bacteriology.

F. Determination of disinfectant activity by agar well diffusion method:

Activity of disinfectants such as Dettol and Phenyl derivative [10], [11] was investigated by Agar well diffusion method [12]. Laboratory cultures of *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were used. Suspension of each isolate was prepared in 5 ml of sterile saline (OD =0.5 at 530 nm) and culture was seeded in Muller-Hinton agar medium. Various dilutions (1:10, 1:20, and 1:30) of Dettol and Phenyl derivative were prepared and 0.1 ml of each dilution were added in the well and incubated at 37^0 C for 24 hrs. The diameter of zone of inhibition around the well was measured and organisms were classified as sensitive or resistant.

III. RESULTS

A. Air flora analysis using Gravity Sedimentation Method:

Air flora of patient's washroom was determined using Gravity Sedimentation Method [8]. The petri plates containing Sterile Nutrient Agar & Sterile Sabouraud's Agar were exposed for duration of 1 min & 5 min (Fig. 1), (Fig. 2). Sedimentation rate (TABLE I) was determined using formula –

$$\text{Sedimentation rate} = \frac{\text{Number of colonies obtained}}{\text{Time of exposure X Area of petri plate}}$$

TABLE I- Average Sedimentation Rate (organisms/m^2/hr.) of air micro flora

HOSPITAL	NO.OF COLONIES				SEDEMENTATION RATE (organisms/m^2/ hr.)	
	Nutrient Agar		Sabourauds Agar		Nutrient Agar	Sabourauds Agar
	1 min	5 min	1 min	5 min		
Government	33	72	8	18	$2.56x10^4$	$5.38x10^4$
Private	24	88	4	25	$19.55x10^4$	$4.23x10^4$

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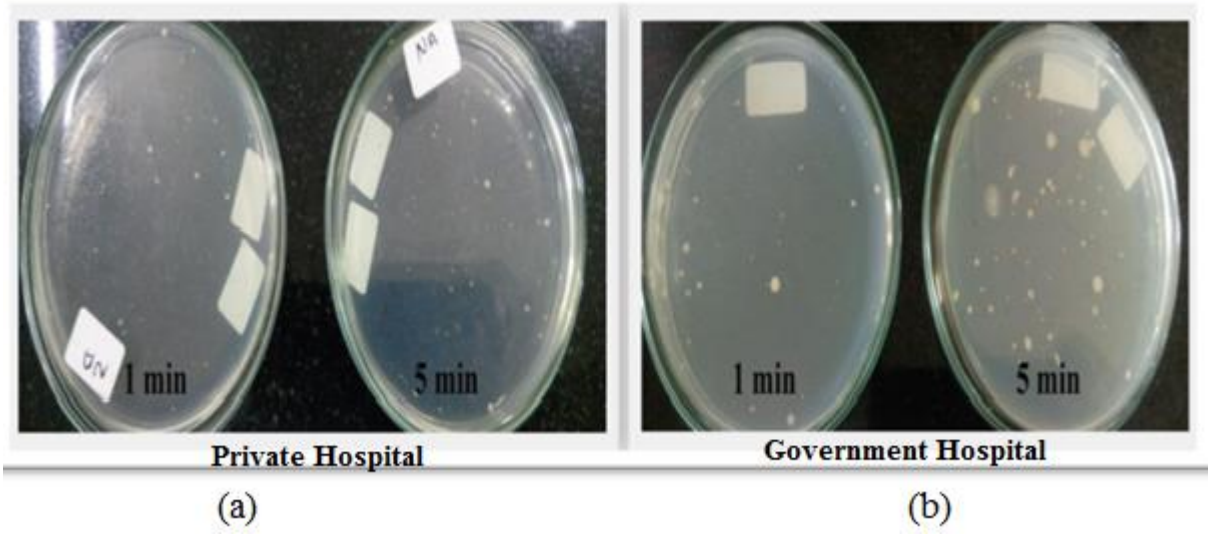

Fig. 1 Enumeration of bacterial air flora using sterile Nutrient Agar plate

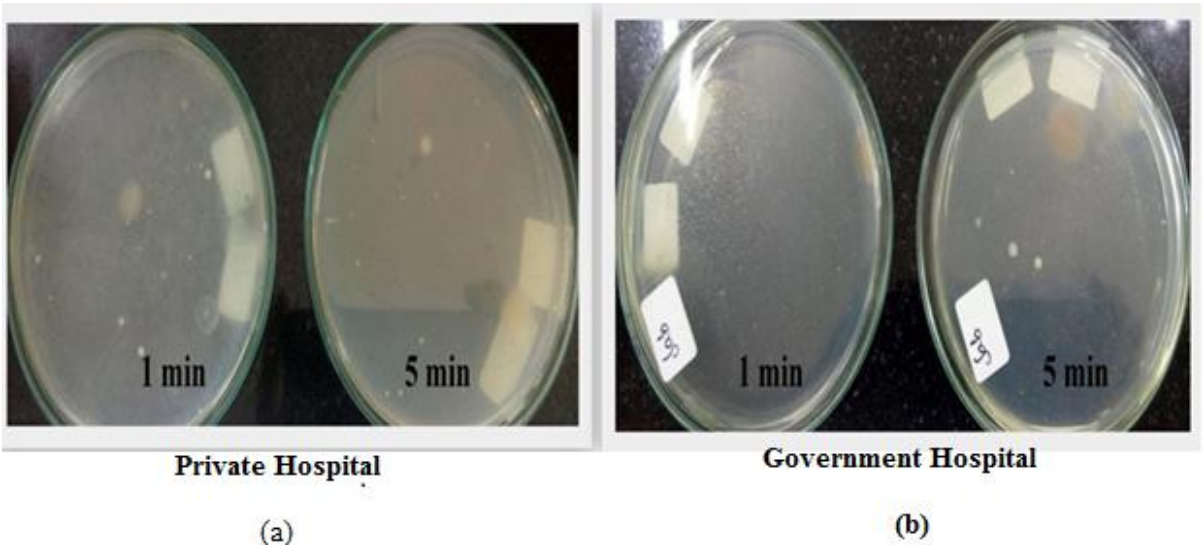

Fig. 2 Enumeration of fungal air flora using sterile Sabourauds' Agar plate

B. Isolation of Organisms from collected samples:

Organisms were isolated from Clinical waste, decomposition room, Dressing tray, General ward, Water reservoir, & Water tap (Fig. 3). The numbers of colonies obtained were as follows (TABLE II):

TABLE II- Number of colonies obtained from each Hospital

Hospital	Number of colonies obtained		
	Gram Positive	Gram negative	Total
Pvt. 1	7	8	15
Pvt. 2	3	12	15
Gvt. 1	6	13	19
Gvt 2	10	8	18

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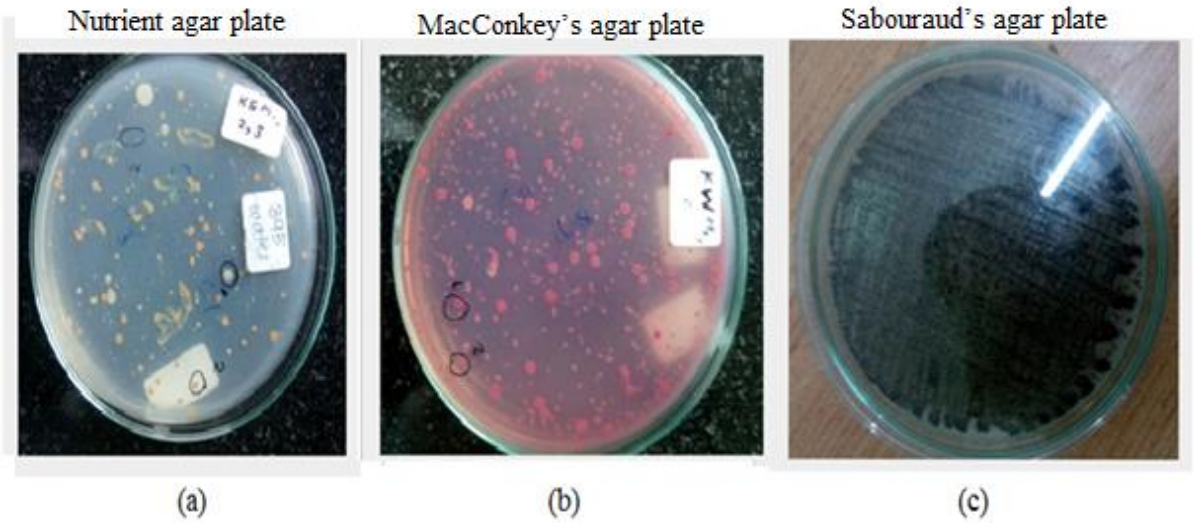

Fig. 3 Isolated organisms from various hospital fomites

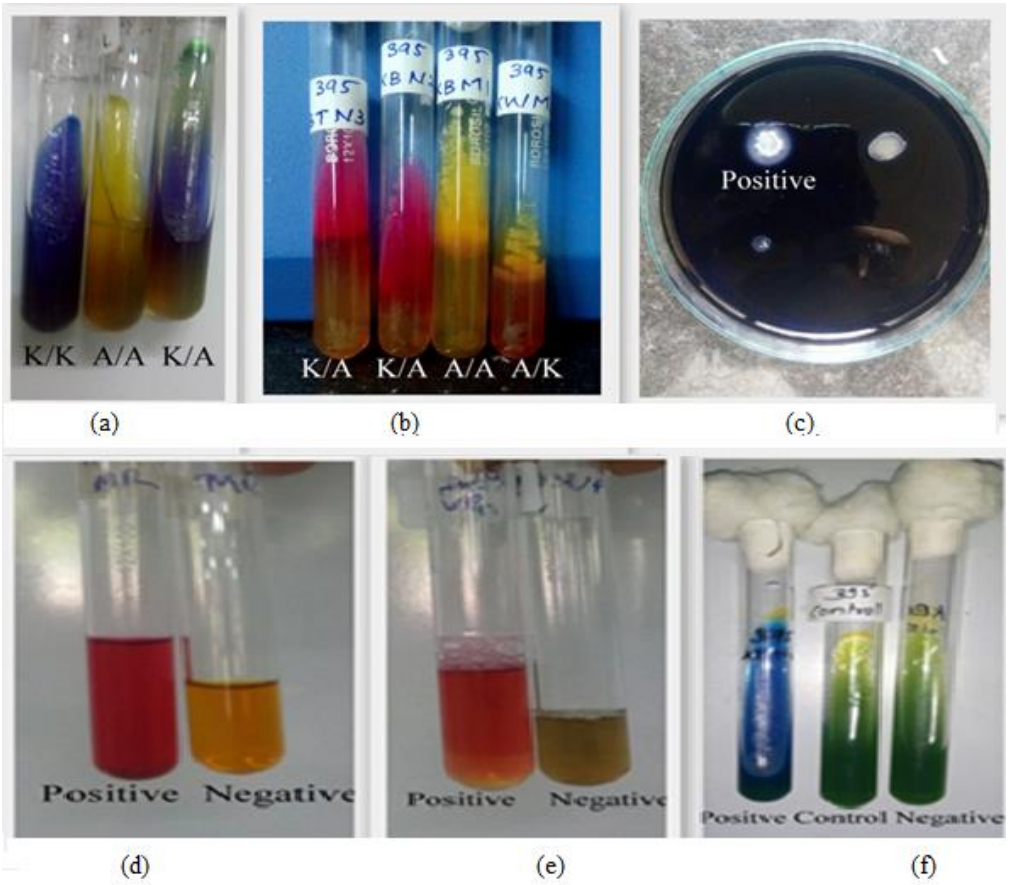

Fig. 4 Biochemical tests of isolates
(a) Lysine Iron agar (b) Triple Sugar Iron agar test (c) Starch Hydrolysis (d) Methyl Red test (e) Vogues-Proskauer test (f) Citrate utilization test

C. Identification of Isolates:

Selective Biochemical tests (Fig. 4) were performed and identification of microbes up to species level was carried out using Bergey's manual (TABLE III), (TABLE IV).

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TABLE III - Micro flora from Private Hospital

TABLE III (A) - Gram Negative Organisms

No.	ORGANISM	BIOCHEMICAL TEST														
		I	MR	VP	Ci	U	SH	MO	O	C	L	Ni	SUGAR			
													G	L	S	M
1	*Actinobacillus ureae*	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-
2	*Escherichia albertii*	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-
3	*Escherichia coli*	+	+	-	-	-	-	+		+	+	+	+	+	+	+
4	*Escherichia vulneris*	-	+	-	-	-	-	+	-	+	+	+	+	+	+	+
5	*Klebsiella mobilis*	-	-	-	+	+	-	+	-	+	+	+	+	-	+	+
6	*Klebsiella oxytoca*	+	-	+	+	+	-	-	-	+	+	+	+	-	+	+
7	*Leminorella richardii*	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
8	*Proteus mirabilis*	-	+	-	+	+	-	+	-	+	-	+	+	-	+	-
9	*Proteus vulgaris*	+	+	-	+	+	-	+	-	+	-	+	+	-	-	+
10	*Providencia alcalifaciens*	+	+	-	+	-	-	+	-	+	-	+	+	-	+	+
11	*Providencia rettgeri*	+	+	-	+	+	-	+	-	+	-	+	+	-	-	+
12	*Tatumella ptyseos*	-	-	+	-	-	-	-	-	+	-	+	+	-	+	+
13	*Yersinia aldovae*	-	+	-	-	+	-	-	-	+	-	+	+	-	-	+
14	*Yersinia fredriksenii*	+	+	-	-	+	-	-	-	+	-	+	+	-	+	+
15	*Yersinia intermedia*	+	+	-	-	+	-	-	-	+	-	+	+	-	+	+
16	*Yersinia kristensenii*	+	+	-	-	+	-	-	-	+	-	+	+	-	-	+
17	*Yersinia pestis*	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+
18	*Yersinia rohdei*	-	+	-	-	+	-	-	-	+	-	+	+	-	+	+

TABLE III (B) - Gram Positive Organisms

No.	ORGANISMS	BIOCHEMICAL TEST									
		U	SH	MO	O	C	E	Ni	MN	COA	GL
1	*Bacillus badius*	-	-	-	+	+	-	-	-	-	-
2	*Bacillus cereus*	-	+	-	-	+	+	+	-	-	-
3	*Bacillus coagulans*	-	+	-	-	+	-	-	-	-	-
4	*Bacillus mycoides*	-	-	-	-	+	+	+	-	-	-
5	*Lysinibacillus sphaericus*	-	-	-	+	+	-	-	-	-	-
6	*Paenibacillus sanguinis*	-	-	-	-	-	+	-	-	-	-
7	*Psychrobacillus insolitus*	-	-	-	+	+	+	-	-	-	-
8	*Staphylococcus aureus*	+	+	-	-	+	-	+	+	+	+

TABLE IV - Micro flora from Government Hospital

TABLE IV (A) - Gram Negative Organisms

No.	ORGANISM	BIOCHEMICAL TEST												SUGAR			
		I	MR	VP	Ci	U	SH	MO	O	C	L	E	Ni	G	L	S	M
1	*Escherichia albertii*	-	+	-	-	-	-	-	-	+	+	-	+	+	+	-	-
2	*Escherichia coli*	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+
3	*Escherichia vulneris*	-	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+
4	*Klebsiella mobilis*	-	-	-	+	+	-	+	-	+	+	+	+	+	-	+	+
5	*Klebsiella oxytoca*	+	-	+	+	+	-	-	-	+	+	+	+	+	-	+	+
6	*Klebsiella pneumonia*	-	-	+	+	+	-	-	-	+	+	+	+	+	-	+	+
7	*Proteus vulgaris*	+	+	-	+	+	-	+	-	+	-	+	+	+	-	+	-
8	*Providencia stuartii*	+	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+
9	*Yersinia enterocolitica*	+	+	-	-	+	-	-	-	+	-	-	+	+	-	+	+
10	*Yersinia fredriksenii*	+	+	-	-	+	-	-	-	+	-	-	+	+	-	+	+
11	*Yersinia pestis*	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	+
12	*Yersinia pseudotubercolosis*	-	+	-	-	+	-	-	-	+	-	-	+	+	-	-	+

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TABLE IV (B) - Gram Positive Organisms

NO	ORGANISMS	BIOCHEMICAL TEST									
		U	SH	MO	O	C	E	Ni	MN	COA	GL
1	*Aneurinibacilus aneurinilytics*	-	-	-	-	+	-	+	-	-	-
2	*Bacillus badius*	-	-	-	+	+	-	-	-	-	-
3	*Bacillus cereus*	-	+	-	-	+	+	+	-	-	-
4	*Bacillus coagulans*	-	+	-	-	+	-	-	-	-	-
5	*Bacillus licheniformis*	-	+	+	+	+	+	+	-	-	+
6	*Bacillus megaterium*	-	+	+	+	+	+	-	-	-	+
7	*Bacillus mycoides*	-	+	-	-	+	+	+	-	-	+
8	*Bacillus pumulis*	-	-	+	-	+	-	-	-	-	+
9	*Bacillus subtilis*	-	+	+	+	+	+	+	+	-	+
10	*Enterococcus faecalis*	-	-	-	-	-	+	+	-	-	-
11	*Enterococcus faecium*	-	-	-	-	-	+	+	-	-	-
12	*Staphylococcus aureus*	+	+	-	-	+	-	+	+	+	+
13	*Staphylococcus epidermidis*	+	-	-	-	+	-	+	-	-	-
14	*Staphylococcus lugdunensis*	-	-	-	-	+	-	+	-	+	-
15	*Staphylococcus pasteuri*	+	-	-	-	-	-	+	+	-	-
16	*Staphylococcus warneri*	+	-	-	-	-	-	+	+	-	-
17	*Streptococcus mutans*	-	+	-	-	-	+	-	+	-	-

KEY:

Symbol	Meaning
+	Positive result
-	Negative result
I	Indole test
MR	Methyl Red test
VP	Vogues-proskauer test
CI	Citrate utilization Test
U	Urea Hydrolysis test
SH	Starch Hydrolysis test
MO	Motility test
O	Oxidase test
C	Catalase Test
L	Lysine Decarboxylase test
Ni	Nitrate Reductase test
E	Esculin test
MN	Mannitol Salt Agar
COA	Coagulase Test
GL	Gelatin Liquefaction
G	Glucose
L	Lactose
S	Sucrose
M	Mannose

D. Determination of disinfectant activity:

To minimize the risk of nosocomial infections, study of organisms' susceptibility to commonly used disinfectants in hospitals is necessary. Hence, Dettol & phenyl derivative disinfectants were selected in this study. Dilutions of 1:10, 1:20 and 1:30 were used and Agar well diffusion method was performed (Fig. 5), (Fig. 6) [12]. 1: 10 dilution of both disinfectant showed inhibition against all selected microbes except *Escherichia coli* (TABLE V), (TABLE VI). Further dilutions of disinfectant showed decreased inhibition.

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TABLE V - Disinfection activity of Dettol

NO.	ORGANISM	DIAMETER OF ZONE OF INHIBITION (mm) AT DIFFERENT DILUTIONS OF DETTOL		
		1:10	1:20	1:30
1.	*Klebsiella pneumoniae*	19	16	9
2.	*Bacillus subtilis*	21	20	16
3.	*Staphylococcus aureus*	17	12	9
4.	*Escherichia coli*	0	0	0

TABLE VI - Disinfection activity of Phenyl derivative

NO.	ORGANISM	DIAMETER OF ZONE OF INHIBITION (mm) AT DIFFERENT DILUTIONS OF PHENYL DERIVATIVE		
		1:10	1:20	1:30
1.	*Klebsiella pneumoniae*	13	10	8
2.	*Bacillus subtilis*	19	16	12
3.	*Staphylococcus aureus*	14	11	9
4.	*Escherichia coli*	0	0	0

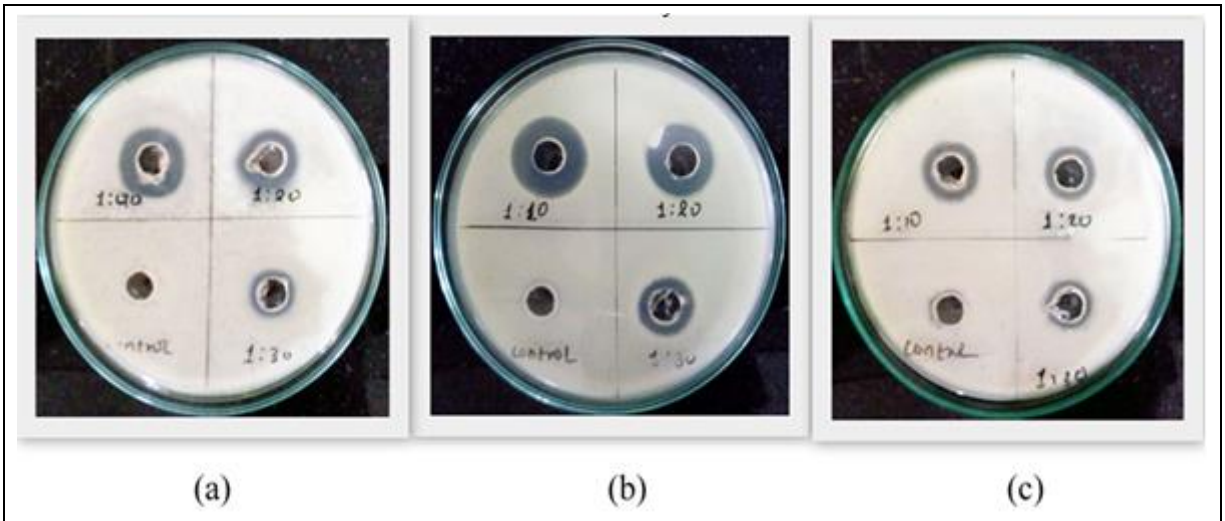

Fig. 5 Disinfectant activity of Dettol on (a) *Klebsiella pneumoniae* (b) *Bacillus subtilis* (c) *Staphylococcus aureus*

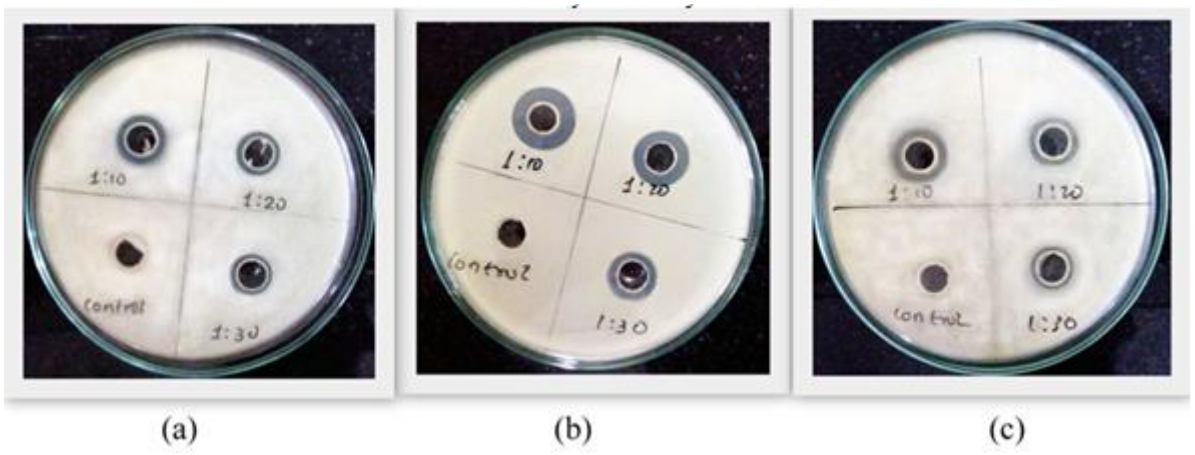

Fig. 6 Disinfectant activity of Phenyl derivative on (a) *Klebsiella pneumoniae* (b) *Bacillus subtilis* (c) *Staphylococcus aureus*

IV. CONCLUSION

Microbial monitoring of hospital environments is important for the quality of Human life. However, hospitalized patients and immunosuppressed patients both are equally prone to acquire Hospital Acquired Infection's (HAI). Air borne infections are most common in individuals with breathing difficulties [7]. Estimation of Airborne bacterial and fungal population from washroom of both Hospital's showed that, Government hospital recorded highest number of bacterial load within '5 Minutes' compared to Private hospital, whereas Private hospital recorded highest number of bacterial load within '1 Minute'. Average airborne fungal population was found to be highest in Private hospital within '5 Minutes' followed by the least count in Government hospital. The study of airborne microorganisms in indoor environment suggest that quality of air depends on various factors such as proper sanitation, frequency of visitor's overcrowding and many more other factors contribute to the quality of air [7]. The high bacterial count recorded in Government hospital could be due to less maintenance of washroom and frequent crowding whereas high fungal count in Private hospital can be due to less efficiency of air sanitization followed in most of the private hospitals.

Research carried out by Bhatia *et. al* [8], they found that children ward, general ward, and casualty of both the hospitals were recorded to have the highest bacterial and fungal load in air. Amongst the results, *Aspergillus spp*. were the most common fungal isolate found along with species of *Fusarium, Mucor, Verticullium, Rhizopus, Penicillium and Candida*. Estimation of 8 different genera were identified in Government hospital whereas 13 different genera in Private hospital. Government hospital reported highest number of Gram positive Bacilli followed by high number of Gram negative short Rods. By these results we can draw a conclusion that Private hospital is better than Government hospital. These can be said because most of the Hospital Acquired Infections include infections caused by Gram positive bacilli. Organisms isolated in both the hospitals were mostly common among which few were of genus *Escherichia, Klebsiella, Providencia, Staphylococcus, Proteus, Yersinia, Bacillus, and Enterococcus. Penicillium, Candida, Mucor, Rhizopus,* and *Aspergillus* were some of the fungal isolates which were common in these four hospitals. Bed and Water reservoir were the two Fomites with highest number of bacterial and fungal population in both the hospitals followed by Waste container which was seen only in Government hospital. Most of the pathogenic organisms were isolated from these areas.

Antiseptics and disinfectants are used extensively in hospitals and other health care Centre's to control the growth of microbes on both living tissues and inanimate objects. They are essential parts of infection control practices and aid in the prevention of Nosocomial infections. But a common problem is the selection of disinfectants and antiseptics because different pathogens vary in their response to different antiseptics or disinfectants. Dettol and phenyl are widely used in hospitals as disinfectants. Dettol and phenyl derivative were selected as disinfectant against three pathogenic bacteria, *Klebsiella spp, Bacillus* and *Staphylococcus spp*. Initially dilutions of 1:100, 1:200 and 1:300 were used which were less effective against these three pathogenic bacteria's. Later dilutions of 1:10, 1:20 and 1:30 were used which showed gradation in zone of diameter of inhibition. Phenyl was less effective against *Klebsiella spp* with the zone of inhibition ranging from 8 to 13 mm. *Bacillus* gave 19 mm zone of inhibition at 1:10 dilutions while *Staphylococcus spp* gave 14 mm zone of inhibition. As the dilution increased the inhibition decreased gradually. With Dettol as disinfectant, *Bacillus* showed less resistant at 1:10 dilution giving 21 mm zone of inhibition. *Klebsiella* and *Staphylococcus spp* showed 19 mm and 17 mm zone of inhibition respectively. As per Research carried out by A. K. Saha *et al.* [11], Sterile Filter paper discs soaked in 5%,10%,25% ,50% & 100% concentration of Dettol, Savlon, Iodine, Phenyl, Formalin and Hydrogen peroxide were selected for experiment against five pathogenic bacteria, *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Klebsiella species* and *Escherichia coli*. Phenyl was least effective against all the pathogens under study while the antimicrobial effect of Dettol was better against *S. aureus, S. typhi* and *E, coli* than against *S. dysenteriae* and *Klebsiella. spp*. In current study, both disinfectants didn't show any inhibition against *Escherichia coli*.

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