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Research Article

Effects of Disinfectants on Microbial Load of Keyboard and Mouse

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Abstract

With the advent use of electronic appliances, the number of computer keyboards and computer mice in use in daily life are increasing. The hands of users are believed to be the main vector for transfer of pathogens in this devices. Disinfectants which are easily accessible and that which have fewer side effects are found to lower the microbial load to some extent. This work systematically investigated the occurrence of a variety of microorganisms on keyboard and mouse. We hypothesized that a variety of bacteria would be isolated from the items and that the various disinfectants such as ethanol and phenolic compounds (Lysol) should eliminate the microorganisms. Similarly, in this study, we investigated the number and nature of contaminating microorganisms on the keyboards and mouse of different locations. Disinfectant-tests to the isolated and identified organisms were done via the phenol coefficient tests where the disinfectants to be used were diluted before. Afore implementing these disinfectants, the microbial load was tallied with the McFarland's broth with the help of colorimeter. Among the six swab samples of keyboards from various locations, the library keyboard had the largest count followed by Classroom2, Classroom 1, Classroom 3, Computer #12 and House. Library mouse, on average, held the largest count followed by Classroom 2, Classroom 3, Computer #12, Classroom 1 and House. The comparative efficacies of the disinfectants were obtained by the two-way ANOVA test. With the calculation from phenol coefficient test, phenol was found to be the most effective.

Keywords: Keyboard; Mouse; Disinfectant; Phenol Coefficient Test; Colorimeter; Two Way Anova

Introduction

Various literatures have revealed that in human environment, microorganisms colonize and contaminate environmental objects in the home, hospital (Brady *et al.*, 2007) schools and day-care environment (Itah and Ben, 2004), and in offices (Bouillard *et al*., 2005). Computer keyboards and mouse are the most open surface parts of computer which show 100% contamination (Chimezie *et al.*, 2013). In various university or educational institutions environment, as the population of facility (internet and e-mails) increases, there is need to recognize that computer equipment may act as a reservoir for the transmission of potential hazardous or pathogenic microorganisms (Hartmann *et al.*, 2004). The ability of a computer to act as fomites has been previously documented in healthcare (Huber and Pelon 2005) and hospital environment (Peppas *et al.*, 2000).

The environmental conditions vary depending on temperatures around the keyboard. Pathogens can also survive on dry inanimate surfaces for month (Kramer, 2006). *Staphylococcus aureus,* usually found on skin or in the nasal environment and surviving only on dry skin outside of the body, appears on keyboards. Organisms like *E.coli, Pseudomonas,* and *Bacillus* spp are also found on the surface of computer keyboard and mouse. Unlike most bacteria, *Enterococcus* spp, a normal flora of bowel, is known to survive adverse conditions in which other bacteria usually cannot grow. *Enterococcus* species represent some of the highest rates of appearances in hospital environment keyboards (Hartman *et al.*, 2004). Facultative anaerobes and anaerobic bacteria along with different species of fungi might also be present on keyboards (Rutala *et al*., 2006).

Given that computers are not routinely disinfected, the opportunity for the transmission of contaminating microorganisms is potentially great (Enemuor *et al.*, 2012). The flora on the keyboard flourishes by utilizing the

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moisture that has been trapped by the dust (Eltablawy and Elhifnawi 2009). The average number of microorganisms present on multiple-user computer keyboards was significantly greater than on single-user keyboards, and the number of keyboards harboring potential pathogens was also greater for multiple- user computers (Anderson and Palombo, 2009).

Disinfectants are chemicals that have the ability to destroy or inhibit the growth of microorganisms. Disinfection is the process of removing microorganisms, including potentially pathogenic ones, from the surfaces of inanimate objects. The British Standards Institutions further defines disinfectants as not killing all microorganisms, but reducing them to level acceptable for a defined purpose, for example, a level, which is harmful neither to health nor to the quality of perishable goods. Chemical disinfectants are capable of different level of actions (Gamage, 2003). Disinfectants are divided into different types on the basis of spectrum of activity (Rao *et al.,* 2014).

High-Level Disinfection (hydrogen peroxide and formaldehyde) kills vegetative microorganisms and inactivates viruses, but not necessarily high numbers of bacterial spores capable of sterilization when the contact time is relatively long (e.g., 6 to 10 hours). They are used for relatively short periods of time (e.g., 10 to 30 minutes).This is supported by specific reports of denaturation of *Escherichia coli* dehydrogenases (Malik and Naeem, 2014).

Intermediate-Level Disinfection (alcohol and hypochlorites) kills vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. Low-Level Disinfection (phenolic disinfectants and quaternary ammonium) kills most vegetative bacteria except *M. tuberculosis*, some fungi, and inactivates some viruses (World Health Organization Laboratory Biosafety Manual 3rd edition, 2004).

The disinfectant to be tested is compared with phenol on a standard microbe. Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective, have a coefficient < 1 (Brewer, 1943).

This study is performed in accordance to demonstrate that microbial contamination of multiple-user computer keyboards may be a common mechanism of transfer of potentially pathogenic bacteria among users. For instance, this present study has endeavored to evaluate the effectiveness of different disinfectants (Lysol, Phenol, Herbal and Ethanol). This study has significant importance in checking whether the selected disinfectant can be implemented to minimize the microbial load of household items.

Materials and Methods

Sample Size

A total of 12 sample from keyboards and mouse (10 samples from open-access locations: library and class rooms from the college and 2 samples from privately owned/ used computer) were processed during the study. 2 control samples from the "out of order"- computer #12 were also included.

Collection of Sample

Convenience method of sampling was used. Selection of sample was made on two bases: Number of individuals using the computer and frequency of use per day. Samples were collected using sterile swabs. The swabs were placed in the sterile plastic covers and transported immediately to the microbiology laboratory (2 minutes away) for serial dilution and immediate culture on the suitable media. In case of delay, the samples are refrigerated at 4°C. Each swab sample was dipped in separate sterile saline tubes, vortexed for homogeneity and serially diluted up to 10^{-7} dilution.

Culture and Isolation of Microorganisms

0.1 ml (100µl) aliquot of different dilutions was cultured into Nutrient Agar plates by Spread plate technique using a sterile glass rod spreader. Cultured plates were incubated for 24 hours at 37°C under aerobic condition. 5ml of 10^{-1} dilution was added to 45 ml of Selenite F Broth to selectively enrich *Salmonella* spp; the cultured broth was incubated only for 7 hours at 37°C and streaked in XLD agar plate.

10^{-1} dilution from each sample was taken for pour plating using selective and differential media like VRBA, Cetrimide agar and MSA to isolate Coliforms, *Pseudomonas aeruginosa* and *Staphylococcus* spp respectively. These plates were incubated for 24 hours at 37°C.

Characterization of Isolates

The identification of bacterial isolates were done using standard microbiological techniques as described in the Bergey's Manual of systemic bacteriology which comprises of studying the colony morphology, Gram's staining reactions and various biochemical properties. Biochemical identification was performed as per standard microbiological procedures.

Disinfectant Efficacy Testing

Turbidity/ Absorbance of McFarland 0.5 was measured using colorimeter at 610 nm. *E. coli* was taken as the reference organism for determining efficacy of disinfectants. The turbidity due to growth of *E. coli* was measured with the absorbance reading of McFarland 0.5 for the same wavelength.

Efficacy of four disinfectants, Ethanol, lysol, Herbal and Phenol were compared using different parameters-

Concentration, Time of exposure required to completely inhibit bacterial growth and phenol coefficient test.

Statistical Analysis

Data (absorbance) obtained in the study were converted to a more sensitive and direct relationship of CFU/ ml using standard "CFU/ ml vs. Absorbance" graph of McFarland. This data was statistically analyzed using 2 way ANOVA test. For uniformity, the level of significance (α) was set at 5%. If calculated value is greater than the tabulated value of α at corresponding degree of freedom (i.e. $F_{cal}>F_{tab}$) then the data is significant, if not the data is insignificant.

Strict quality control was maintained to obtain reliable microbiological results. For reliability, the entire procedure was repeated at least twice or more to obtain consistent results. More than one colorimeter was used to reduce non-sampling errors.

Results and Discussions

A total of 12 swab samples were collected from 6 desktops (6 corresponding keyboards and 6 computer mice) among which the multi-user computers, in general, were found to have higher microbial population than single user computer.

Enumeration of Aerobic Mesophilic Bacteria from Keyboard and Computer mice

Among the six swab samples of keyboards the library keyboard had the largest bacterial count of 2.11 x 10^7 CFU/ ml followed by Classroom 2 (1.97 x 10^7 CFU/ ml), Clasroom1 (1.55 x 10^7 CFU/ ml), Classroom 3 (1.22 x10^7 CFU/ ml), Computer # 12 (1.12 x10^7 CFU/ ml) and House (5.05 x 10^6 CFU/ml) as shown in Table 1. Similarly, mean aerobic mesophilic bacterial count of compter mice , in general, was higher than the keyboards of the corresponding locations. Library mouse, on average, held the largest count of 2.28 x 10^7 CFU/ ml followed by Classroom 2 (2.55 x 10^6CFU/ ml), Classroom 3 (1.76 x 10^7 CFU/ ml), Computer # 12 (1.36 x 10^7 CFU/ml), Classroom 1 (1.06 x 10^7 CFU/ ml) and House (5 x 10^5 CFU/ ml) which is shown in Table 2.

Identification of the Isolated Colonies

The size, shape, margin and elevation of the colonies were observed. Gram staining and microscopy revealed the isolated colonies in VRBA agar plate and in Cetrimide agar plates to be gram negative rod, while those on 10^{-7} NA plate and MSA plate to be gram positive rod and gram positive cocci respectively. Based on biochemical tests, the presumed Coliform was identified to be *E. coli*, presumed *Staphylococcus* spp, *Pseudomonas aeruginosa* and *Bacillus* spp were respectively identified as *S. aureus and* CoNS, *Pseudomonas aeruginosa and Bacillus* spp (Table 3). Similar findings have been reported in the studies of Rutala *et al*. (2006), Eniola and Livingstone (2013).

Table 1: Enumeration of Aerobic mesophilic bacteria from Keyboard

Location	Dilution	Keyboard		
		Colonies	CFU/ ml	Mean CFU/ ml
Library	10^{-5} 10^{-6} 10^{-7}	52 18 4	5.2 x 10^6 1.8 x 10^7 4 x 10^7	2.11 x 10^7
Classroom 1	10^{-5} 10^{-6} 10^{-7}	44 12 3	4.4 x 10^6 1.2 x 10^7 3 x 10^7	1.55 x 10^7
Classroom 2	10^{-5} 10^{-6} 10^{-7}	50 14 4	5.0 x 10^6 1.4 x 10^7 4 x 10^7	1.97 x 10^7
Classroom 3	10^{-5} 10^{-6} 10^{-7}	37 13 2	3.7 x 10^6 1.3 x 10^7 2 x 10^7	1.22 x 10^7
Computer # 12 (control)	10-5 10-6 10^{-7}	26 11 2	2.6 x 10^6 1.1 x 10^7 2 x 10^7	1.12 x 10^7
House	10^{-5} 10^{-6}	21 8	2.1 x 10^6 8 x 10^6	5.05 x 10^6

Table 2: Enumeration of bacterial load in compter mice

Location	**Dilution**	**Mouse**		
		Colonies	**CFU/ ml**	**Mean CFU/ ml**
Library	10^{-5} 10^{-6} 10^{-7}	24 16 5	2.4×10^{6} 1.6×10^{7} 5×10^{7}	2.28×10^{7}
Classroom 1	10^{-5} 10^{-6} 10^{-7}	18 10 2	1.8×10^{6} 1.0×10^{7} 2×10^{7}	1.06×10^{7}
Classroom 2	10^{-5} 10^{-6} 10^{-7}	11 4 ---	1.1×10^{6} 4.0×10^{6}	2.55×10^{6}
Classroom 3	10^{-5} 10^{-6} 10^{-7}	7 2 5	7.0×10^{5} 2.0×10^{6} 5×10^{7}	1.76×10^{7}
Computer # 12	10^{-5} 10^{-6} 10^{-7}	17 9 3	1.7×10^{6} 9.0×10^{6} 3×10^{7}	1.36×10^{7}
House	10^{-5} 10^{-6} 10^{-7}	5 --- ---	5×10^{5}	5×10^{5}

Table 3: Identification of the Isolated Colonies

Biochemical tests/ Features	*E.coli*	*Bacillus* spp	*Pseudomonas aeruginosa*
Shape	Rod	Rod	Rod
Arrangement	Cluster/palisade	Cluster	Often in chain of 2
Gram's stain	-	-	-
Tryptophan hydrolysis	+	-	-
H_2S production	-	-	-
Motility	+	+	-
Methyl Red	+	-	-
Vogues-Proskauer	-	+	+
Citrate Utilization	-	+	+
TSIA	A/ A G	A/ NC	ALK /NC
Oxidative/Fermentative	Both	Oxidative	Oxidative
Catalase	+	+	+
Oxidase	-	-	+
Coagulase	-	-	-
Gelatin hydrolysis	-	+	+

Disinfectant Efficacy Test

The disinfectant activity was studied on *E. coli* as reference organism. Two variable parameters: Concentration of disinfectant and time period of exposure and a single independent variable: bacterial load corresponding to absorbance of 0.5 McFarland (at 610 nm) has been taken into account to determine disinfectant efficacy. For every sample, initial absorbance was measured just before incubation; this absorbance was taken as reference for determining whether growth has been inhibited by action of disinfectant or not.

The disinfectant power of each disinfectant was expressed as "phenol coefficient", in which the disinfectant power of disinfectants was compared to that of phenol.

Disinfectant Efficacy Test for Ethanol

The initial absorbance value (0.13) was taken as the reference value. 70%, 90% and 100% ethanol were found to be effective for inhibiting bacterial growth at exposure periods greater than 5 minutes. 70% ethanol was found to be the most effective as it inhibited bacterial growth before 5 minutes. 30 and 50 % ethanol were found to be least effective as growth was observed even after 10 minutes of exposure. The absorbance readings for different concentrations of ethanol for different time periods of exposure is depicted in the Fig. 1.

Disinfectant efficacy test for Lysol

The initial absorbance value (0.26) was taken as the reference value. Lysol in concentrations 1/50 or more was

required for inhibiting bacterial growth within 7.5 minutes of exposure. Dilutions greater than 1/50 also exhibited inhibition, although none could completely halt growth. All in all, the greater degree of inhibition by Lysol does prove it to be more effective than ethanol. The absorbance readings for different concentrations of lysol for different time periods of exposure are given in Fig. 2.

Disinfectant efficacy for Phenol

The data shown in Fig. 3 shows that initial absorbance value (0.17) was taken as the reference value. Higher concentration (1/50) of phenol succeeded in completely inhibiting the growth while higher dilutions (1/120 and 1/150) were not effective in completely halting the growth. As for phenol, the concentration 1/100 gave the most valid reading for determining the phenol coefficients.

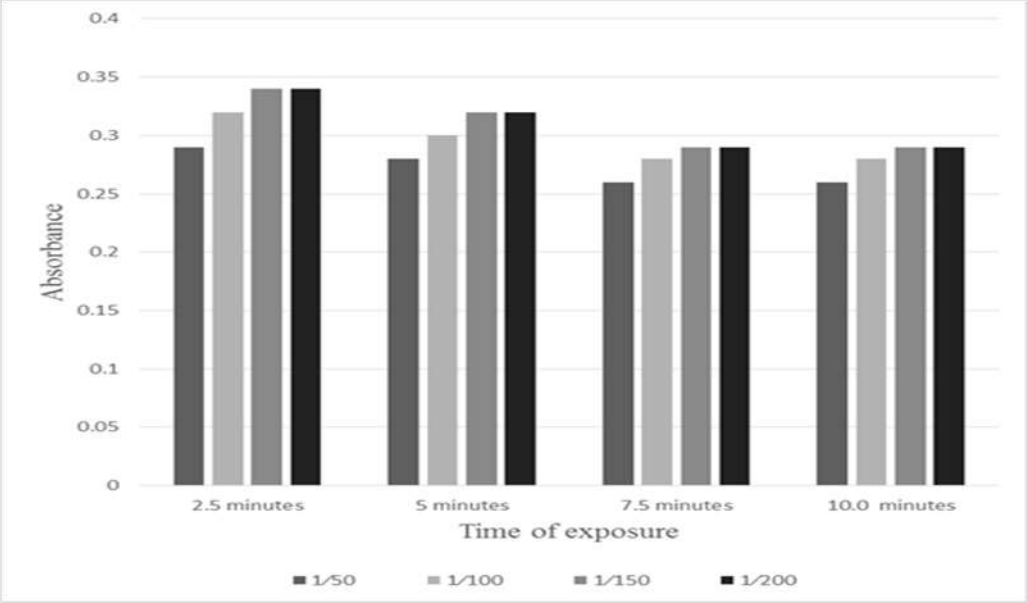

Fig. 1: Disinfectant efficacy test for Ethanol; Constant value =0.13 (- or no growth) values> 0.13= growth

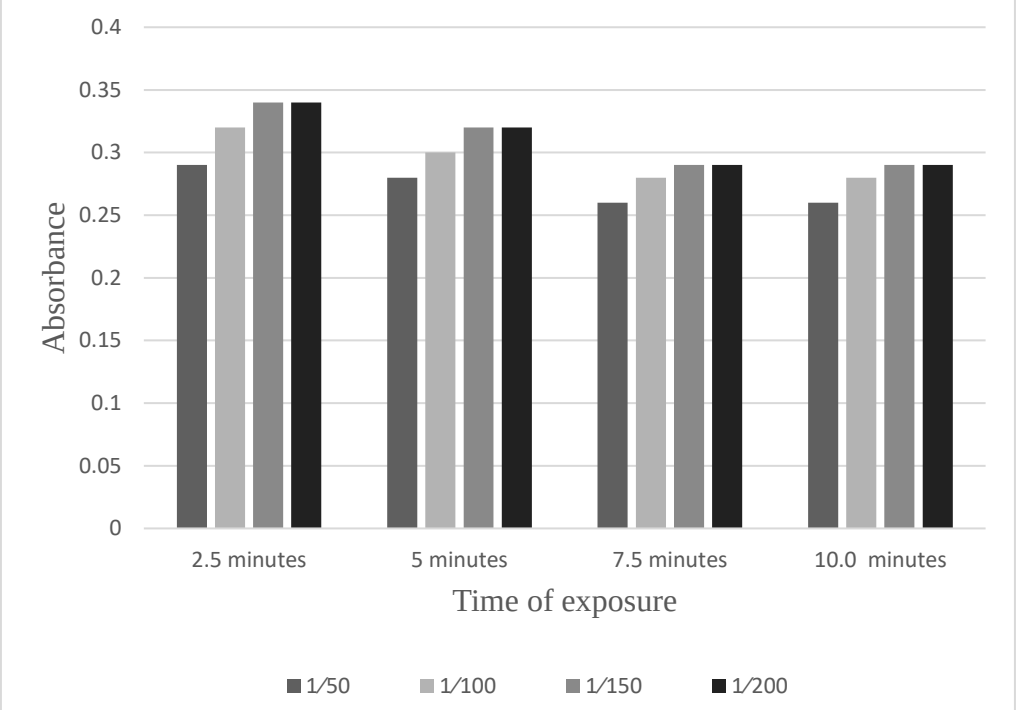

Fig. 2: Disinfectant efficacy test for Lysol; Constant value =0.26 (- or no growth) values> 0.26= growth

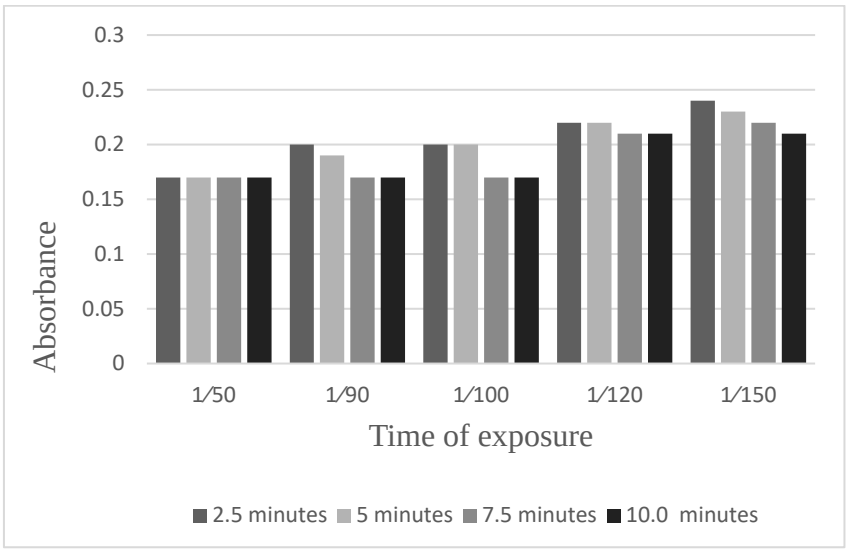

Fig. 3: Disinfectant efficacy test for Phenol; Constant value =0.17(- or no growth) values> 0.17= growth

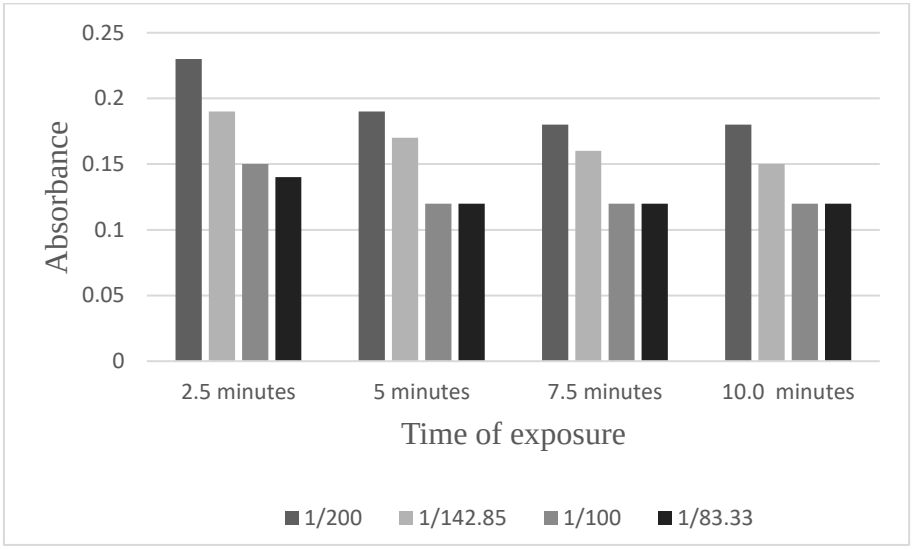

Fig. 4: Disinfectant efficacy test for herbal disinfectant ; Constant value =0.12 (- or no growth) values> 0.12= growth

Disinfectant efficacy for Herbal disinfectant

The initial absorbance value 0.12 was taken as reference. The disinfectant efficacy of Herbal disinfectant coincided with that of phenol, with it showing maximum effectiveness at concentration 1/100. Lower concentrations 1/200 and1/142.5 were not effective (Fig. 4).

Tentative Phenol coefficients of the disinfectants used

Among the different disinfectants, the Herbal disinfectant had phenol coefficient equal to 1, while Lysol and ethanol had phenol coefficients 0.5 and 0.11respectively (Table 4).

Table 4: Phenol coefficients of disinfectants

Disinfectant	**Concentration (v/V)**	**Reference Absorbance**	**2.5 mins**	**5 mins**	**7.5 mins**	**10.0 mins**	**Phenol Coefficient**
Phenol	1/100	0.17	0.20 (+)	0.20 (+)	0.17 (-)	0.17 (-)	
Lysol	1/50	0.26	0.29 (+)	0.28 (+)	0.26(-)	0.26(-)	0.5
Ethanol	90/100	0.13	0.19 (+)	0.15 (+)	0.13(-)	0.13(-)	0.011
Herbal (best fit data)	1/100	0.12	0.15 (+)	0.12 (-)	0.12(-)	0.12(-)	1

Comparison of F ratio from Two way ANOVA Test

F ratio obtained by Two way ANOVA test is significant for every disinfectant. The absorbance data obtained was analyzed by two way ANOVA test for determining the effectiveness of individual disinfectant. Two way ANOVA test was preferred because of its ability to explore variances within subgroups of the same sample.

In all four disinfectants, the F_{cal} was found to be greater than F_{tab} at 5% level of significance for corresponding degrees of freedom. Thus, in every case, alternative hypothesis was accepted, meaning that there is significant difference in the CFU/ ml due to different concentrations and also due to different time periods of exposure. From the data it can be concluded that it is the concentration of lysol that plays a more significant role in inhibiting the microbial growth. In others (ethanol, Phenol and herbal disinfectant) the F ratio of time exposure was greater than the F ratio of concentration, for these it is the time period of exposure that play an important role in inhibiting bacterial growth.

Table 5: Two way ANOVA Test results

S.N.	Disinfectant	F ratio	D.f	F cal	Result
1.	Lysol	Con: 83.01	(3,9)	3.86	Significant
		Time: 63.84	(3,9)	3.86	Significant
2.	Phenol	Con: 7.19	(3,12)	3.49	Significant
		Time: 31.84	(4,12)	3.26	Significant
3.	Herbal	Con: 33.61	(3,9)	3.86	Significant
		Time: 135.78	(3,9)	3.86	Significant
4.	Ethanol	Con: 5.56	(3,12)	3.49	Significant
		Time: 7.77	(4,12)	3.26	Significant

Conclusion

The study indicated high levels of bacterial load in the computer keyboards and mouse with the open access computers (such as library computers) bearing bacterial load up to 2.11 x 10^7 CFU/ ml (keyboard) and 2.28 x 10^7 (mouse). Privately owned and singly used computers, on other hand, had bacterial load up to 5.05 x 10^6 CFU/ ml (keyboard) and 5 x 10^5 (mouse) which is more than 40 times less. The findings of the study clearly revealed that the multi-user computer had higher bacterial load than the single-user computer. The alikeness in bacterial load between keyboard and mouse, reveals that mouse have higher bacterial density than the keyboards. The study also verified the classical concentration vs. time of exposure relationship of chemical disinfectants. As to our experiment, ethanol was found to be the least effective while phenol and herbal disinfectant were found to be the most effective. The final verdict for the most effective was given to the herbal disinfectant for it exhibited more effect than phenol for same concentration. The study revealed that the "Herbal" disinfectant which is being used in the native concentration (1/142.85) is neither effective nor potent enough to inhibit *E. coli* growth. Phenol coefficient test revealed that the same disinfectant in 1/100 concentration is effective in inhibiting the *E. coli* growth.

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