



THE POTENCY OF COMMONLY USED DISINFECTANTS ON BACTERIA ISOLATED FROM IN-ANIMATE SURFACES IN THE MICROBIOLOGY LABORATORY, UMARU MUSA YARDUA UNIVERSITY KATSINA

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ABSTRACT

Disinfection is the process of removing microorganisms including potentially pathogenic ones from the surface of in-animate objects. The ubiquity of microorganisms has implicated door knobs, human hands, desks surfaces etc. to be reservoirs of microorganisms. This study analyses three (3) samples from five (5) inanimate surfaces of the Microbiology laboratory, three different organisms were isolated and identified using series of biochemical tests, phenol coefficient and surface disinfection test were also carried out. The phenol coefficient test involve exposure of the isolates to different dilution of test disinfectants to determine the highest dilution factor that inhibit microbial growth while surface disinfection test involved the use of soiled slides to test the potency of the test disinfectants on the isolates. The organisms isolated were Staphylococcus aureus, Klebsiella sp. and E. coli. Klebsiella sp. showed the highest resistance to the test disinfectants than E. coli and S. aureus. The result of this study confirms the contamination of inanimate surfaces with variety of pathogenic bacteria (S. aureus 25%, E. coli 23% and Klebsiella sp. 52%). Disinfectant A had the highest phenol coefficient of 6.0, C had 4.0 and disinfectant B had the lowest phenol coefficient of 3.0. Microorganisms live as transient contaminants in fomites where they constitute a major health hazards as sources of community acquired infections. Therefore, constant and regular disinfection should always be conducted at different concentrations so as to determine the potent concentration against the adhered microorganisms on various surfaces of the laboratory.

Keywords: Disinfection, Phenol coefficient, Surface disinfection, Contamination, Inanimate surfaces.

INTRODUCTION

Disinfectants are chemicals having the ability to destroy or inhibit the growth of microorganisms. Disinfection is the process of removing microorganisms including potentially pathogenic ones, from the surface of in-animate objects (Aboh *et al.*, 2013).

Disinfection is sometimes mistaken for sterilization but differences between the two concepts lies on the fact that disinfection unlike sterilization is normally associated with the killing of vegetative forms of microorganisms and not necessarily their spore (Oluwayemisi *et al.*, 2012).

Potency is the strength or quality of a substance (Encarta, 2009). It is a measure of a substance activity expressed in terms of the amount required to produce an effect of given intensity.

The concept of testing disinfectants or checking disinfectant potency is very old. Robert Koch described a disinfectant test in the article '*uber desinfektion*' in 1881. A silk thread was contaminated by submersion in a liquid culture

of *Bacillus anthracis*. After drying, the contaminated thread was immersed in several disinfectants solution for a given exposure time. The thread was then cultured in a nutrient broth and no growth after incubation indicated activity of the disinfectant. He concluded from the comparisons of disinfectant solutions that mercuric chloride was the most active disinfectant (Reybrouck, 1998).

The ubiquity of microorganisms has implicated door knobs, human hands, desks surfaces e.t.c to be reservoirs of a lot of microorganisms. In a study reported on the hygiene of environmental surfaces, it was shown that the most implicated sources of infection is door handles of toilets and bathrooms (Reynold, 2005).

A research on the bacterial public health hazard in the public female restrooms at Taif, kingdom of Saudi Arabia, reported that; out of the 187 specimens collected by surface swabs, 71.9% were positive for bacteria. 39% of the positive specimens were samples from door knobs (Sherifa *et al.*, 2013).

The presence of these bacteria on surfaces is really a timed bomb of epidemiological concern. The use of disinfectants, to eliminate or reduce the populations of such bacteria from such surfaces is essential since the use of disinfectants is mostly employed on inanimate surfaces. This will help to reduce the level of microbial contamination (Maris, 1995).

The need to test for the potency of disinfectants prior to use is as important as the use of the disinfectant itself. The selection of disinfectants should be based on the activity they are expected to carry out and not necessarily on sales pitch nor on what one has always used. Disinfectants should be tested periodically to ascertain their potency (Oluwayemisi *et al.*, 2012).

Objectives

- (1) To isolate bacteria from in-animate surfaces using microbiological procedures.
- (2) To determine the phenol coefficient of commonly used disinfectants using the Rideal-Walker test.
- (3) To conduct a surface disinfection test to assess the effectiveness of the disinfectants against surface adhered bacteria.

MATERIALS AND METHODS

The research was conducted in the microbiology laboratory, Umaru Musa Yar'dua University, Katsina. Fifteen (15) samples were collected using sterile cotton swabs from bench surfaces, door knob (back and front), incubator, sink and faucet surfaces of the laboratory.

The test disinfectants were Ethanol, Methylated spirit and Detol. The commonly used disinfectants were renamed for the purpose of this research as disinfectant A, B and C respectively.

The swabs were pre-enriched in sterile peptone water and were incubated at 37°C for 24hrs. After incubation period, samples that showed evidences of growth were streaked onto prepared Eosin Methylene Blue and mannitol salt agar which were all labeled against the source of each specimen followed by incubation at 37°C for 24 hours. After 24 hours, the plates were observed and the colonial morphology were recorded. Pure culture of the isolates were also obtained and a colony from each of the pure cultures was Gram stained and subjected to series of biochemical tests. Gram positive isolates were subjected to catalase,

oxidase and coagulase test while Gram negative isolates were subjected to IMViC Test i.e. Indole, Methyl-red/Voges Proskauer test and Simmon Citrate Utilization test.

Disinfectant test

Prior to disinfectant test, the method of El-mahmood and doughari, (2009) was used to standardize inocula and the Rideal-Walker Phenol Coefficient Test was used to determine the phenol coefficient of the disinfectants as the ratio of the reciprocal of the highest dilution of disinfectant that inhibits the growth of the isolates at 10 minutes.

Surface disinfection test

Zero point five (0.5ml) from the standardized inoculum was smeared on sterile slides each for a test disinfectant. 0.5ml of the diluted disinfectants was dropped and spread with a sterile wooden applicator stick on the surfaces of the dry slides. They were allowed to stay for 10 minutes. Duplicate slides for each organism was made to serve as a control, where 0.5ml of sterile distilled water was dropped and spread on the surface of the slide the same way the disinfectant was spread and were allowed for 10 minutes. The slides were washed off with 0.5ml sterile water into a sterile petri dish and mixed properly. Half (0.5)ml was pipetted and with a bent glass rod it was spread on a nutrient agar plates. All the Plates were incubated at 37°C for 24 hours. The number of colonies were counted and compared with the control to evaluate the effectiveness of each of the test disinfectant as compared to distilled water (Reybrouck, 1998).

RESULTS

The organisms isolated were *Staphylococcus aureus* (25%), *Eschericia coli* (23%) and *Klebsiella sp.* (52%).

The results of the phenol coefficient test of the various disinfectants showed that all the disinfectants were potent against the isolated organisms. Comparatively, some of the disinfectants were potent at higher dilution than others as seen in the phenol coefficients of each of the disinfectants (table 1) Disinfectant A had the highest phenol coefficient 6.0. Disinfectant B and C had phenol coefficients of 3.0 and 4.0 respectively. The result of the phenol coefficients of Disinfectant A in this study agrees with a previous report (Aboh *et al.*, 2013).

TABLE 1: Phenol coefficient of test disinfectants

DISINFECTANT	DILUTION	TIME			PHENOL COEFFICIENT	ISOLATES
		5 Minutes	10 Minutes	15 Minutes		
PHENOL	25:90	NG	NG	NG		
	50:95	NG	NG	NG		
	100:100*	G	NG	NG		
DISINFECTANT A	25:400	NG	NG	NG	6.0	<i>Klebsiella sp.</i>
	50:500	NG	NG	NG		
	100:600*	NG	NG	NG		
DISINFECTANT B	25: 200	NG	NG	NG	3.0	<i>E. coli</i>
	50:300*	NG	NG	NG		
	100:400	G	G	NG		
DISINFECTANT C	25:200	NG	NG	NG	4.0	<i>S. aureus</i>
	50:300	G	NG	NG		
	100:400*	G	NG	NG		

Key:

Phenol coefficient = $\frac{\text{Dilution of test disinfectant that kills test organism at time 10mins (*)}}{\text{Dilution of Phenol that kills test organism at time 10mins (*)}}$

+ = Positive, - = Negative, * = Critical dilution for phenol coefficient, NG= No growth, and G=growth

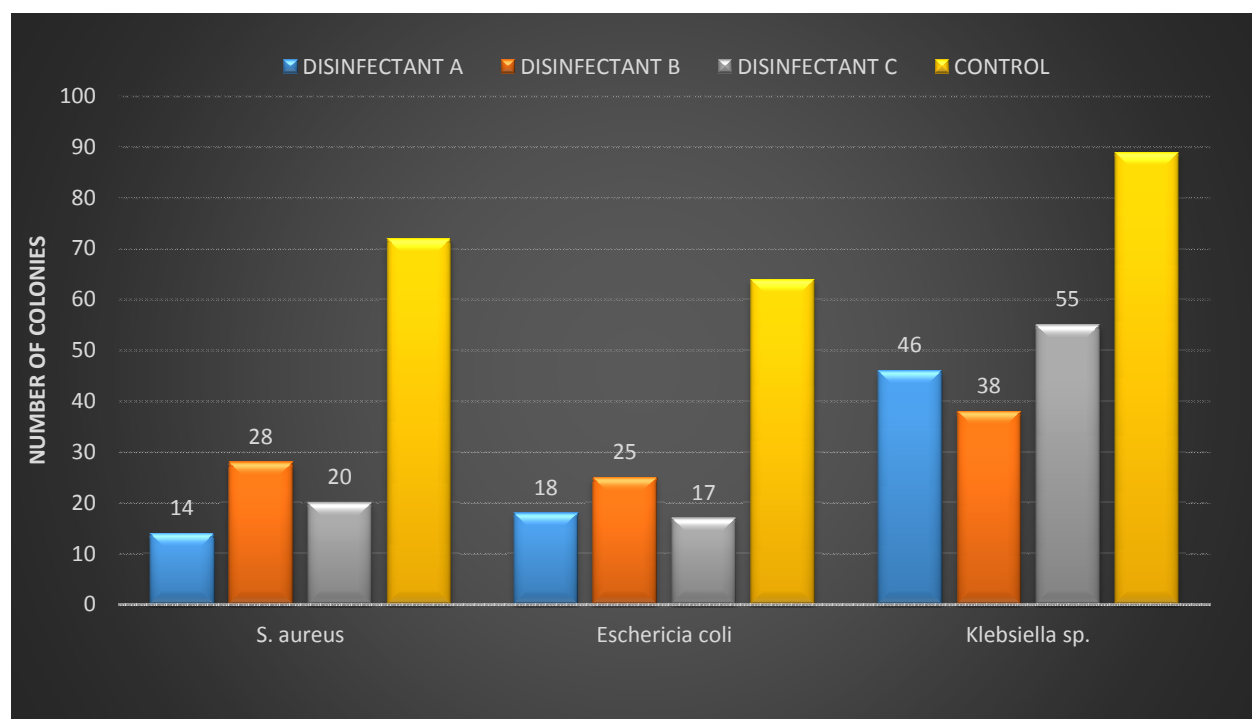


Fig.1 The effect of disinfectants against the test isolates.

For *S. aureus*, the control (distilled water) had 72(54%) colonies of surviving isolates as counted on plates after incubation period. Comparing this control to each of the test disinfectants, disinfectant A had the highest

effect as only 14(10%) colony of *S. aureus* were obtained after the treatment, while Disinfectant B and C, 28(21%) and 20(15%) colonies were counted after the treatments respectively.

For *E. coli*, 64(51%) colonies were counted after treatment with the control. Disinfectant C had the highest effect as only 17 colonies (14%) survived after treatment with the disinfectant. For Disinfectants A and B, 18(15%) and 25(20%) colonies respectively were counted after the treatments.

Klebsiella sp. showed the highest resistance to treatment with the disinfectants. After treatment with the control, 89(39%) colonies of the organism survived. Disinfectant B had the highest effect as 38(17%) colonies were counted. For Disinfectant A and C, 46(20%) and 55(24%) colonies respectively were obtained after the treatment.

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CONCLUSION

The result of this research confirms the contamination of in-animate surfaces with variety of pathogenic bacteria including *S. aureus* (25%), *E. coli* (23%) and *Klebsiella sp.* (52%) and different disinfectants worked best at different dilutions. All the disinfectants were potent against the isolated bacteria but some exhibited greater effects at higher dilutions than others. Disinfectant A had the highest phenol coefficient of 6.0, disinfectant C had 4.0 and disinfectant B with phenol coefficient of 3.0. The greatest resistance to the activity of the various disinfectants was observed by *Klebsiella sp.*

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