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A Study on the Effectiveness of Domestic Disinfectants against Some Human Pathogens

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The efficiency of four disinfectants.(lizol, domex, phenyl (Black cat) and stericlean) was tested against two gram positive (one *S aureus* ATCC 6538 strain and one clinical isolate of *S aureus)* and seven gram negative (one ATCC strain i.e. *Vibrio cholerae* ATCC 210 and six clinical isolates) organisms by phenol coefficient method (Rideal-Walker method, 1903) ; so far the standard test for evaluating most antiseptics and disinfectants of household use. It was observed that all the disinfectants used were highly effective against *S. aureus* ATCC 6538 and less so for *S. aureus* clinical isolates. Again out of all the gram negative organisms tested lizol was highly effective against *Shigella flexneri* and less as to others. Out of the gram negative organisms tested, when a comparative study was made, domex was found to be less effective against *Proteus mirabilis* and highly effective against *Salmonella typhi.* Phenyle (black cat) was highly effective against *Shigella flexneri* and less effective to *Klebsiella pneumoniae* but stericlean was more effective towards *Proteus mirabilis* and less so against *Pseudomonas aeruginosa.*

Key words: Domestic disinfectants, Phenol Coefficient method, Human pathogens

Diseases are caused by microorganisms which are ubiquitous. Getting sickness from a microorganism can happen in an unhealthy environment. They adopt various means to enter our body and we being the most developed creature in the universe use various methods to get rid of them. Health is wealth and using a disinfectant to kill bacteria is an important way to stay healthy. Disinfectants are liquid substances that kill germs or bacteria on nonliving objects. They are usually chemicals used to clean clothes, rooms, toilets, dishes and sewer system to help stop city wide diseases, used to help hospitals and care units to stay clean and free from germs. Mainly the disinfectants contain chemicals like alcohols, aldehydes (formaldehyde and glutaraldehyde), hypochlorites, iodophores, phenols, pine oil disinfectant and quaternary ammonium compounds. In the present study four disinfectants (lizol, phenyl, domex and stericlean) were evaluated for their antimicrobial property on some human pathogen depending on their antimicrobial property or some human pathogen depending on their local demand by phenol coefficient method (Rideal and Walker, 1903), sometimes called the FDA (Food and Drug Administration) method for testing disinfectants miscible with water and exerting their action in a manner similar to that of phenol.

MATERIALS AND METHODS

Disinfectants used in the study

Four disinfectants were evaluated for their antimicrobial property on human pathogens depending on their local demand. These include

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lizol, phenyl (Black cat), domex and stericlean (\Modicare).

Microorganims used

The microorganisms used include both gram positive and gram negative bacteria.

Gram +ve microorganisms used are *Staphylococcus aureus ATCC 6538* and *Staphylococcus aureus* (Clinical isolates).

Gram –ve microorganisms were *Salmonella typhi, Vibrio cholerae* (ATCC 270), *Pseudomonas aerugenosa, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis* and *Shigella flexneri.*

The standard strains were obtained from ATCC (American Type Culture Collection) and the pathogens were isolates of clinical samples, isolated and identified in P.G .Department of Microbiology, O.U.A.T, Bhubaneswar.

Pure cultures of all the above standard and test strains were made by sub culturing the organisms from the previously maintained slant (NA slant) in duplicate and incubated at 37°c for 24 hours. One set was used and another set was preserved for further use.

The effectiveness of the above domestic disinfectants was determined by phenol coefficient

Table 1. Phenol coefficient of different disinfectants

Organism	Disinfectants			
	Llizol	Phenyl	Domex	Stericlean
Staphylococcus aureus (ATCC 6538)	10.52	1.05	4.21	13.68
Staphylococcus aureus	8.42	4.24	2.1	18.94
Salmonella typhi	5.55	0.11	8.88	12.22
Vibrio cholerae ATCC 270	4	0.5	8	11.5
Pseudomonas aeruginosa	0.66	0.06	4	7
Klebsiella kpeumonae	6	0.05	2.66	13.33
E.coli	0.11	0.04	10.1	13.33
Proteus mirabilis	0.5	0.1	2	18
Shigella flexineri	5.56	0.55	4.44	15.5

among the row F < 0.05 (Insignificant)
among the column F> 0.05 (Significant)

Table 2. Antibiogram of gram positive organisms

Organisms	Zone of inhibition (in mm)											
	Va	A	E	Cdm	M	NX	Cd	Cf	Lz	P	Am	T
Staphylococcus aureus ATCC 6538	18	37	13	31	26	31	31	25	12	27	35	30
Staphylococcus aureus	18	10	34	25	10	12	30	18	34	20	11	32

> 12 = Sensitive, < 12 = Resistant

Table 3. Antibiogram of gram negative organisms

Organisms	Zone of inhibition (in mm)											
	P	Nx	Pt	T	G	Va	Cf	Ca	Sc	C	Lo	Nf
Salmonella typhi	R	34	20	10	22	R	R	40	20	R	23	16
Vibrio cholerae (ATCC270)	R	38	34	15	27	R	R	R	10	23	28	10
Pseudomonas aeruginosa	R	28	24	26	20	R	R	22	13	R	R	18
Klebsiella pneumoniae	R	29	28	12	22	R	R	19	28	13	21	R
Escheirichia coli	R	33	32	38	31	23	35	R	40	31	34	36
Protues mirabilis	R	31	36	38	24	22	24	12	30	33	23	40
Shigella flexneri	R	40	32	20	28	R	40	35	31	34	36	16

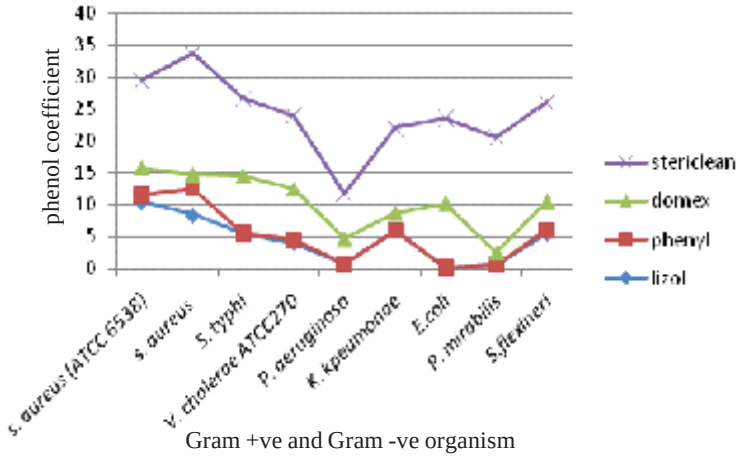

Fig. 1. Graph showing different phenol coefficient of different disinfectants

method (Rideal-Walker method, 1903). Different dilutions of phenol were prepared (i.e. 1:95, 1:100, 1:110, and 1:150). The dilutions were made accurately and within the limitation of the test. The different dilutions were prepared with distilled water in sterilized conical flasks and 5 ml was transferred to each tubes. In the same way different dilutions of disinfectants were prepared and transferred to another set of tubes. To each dilution of disinfectants and phenol, 0.5 ml (500µl) of 24 hours broth culture of the standard and test organisms was added aseptically. Care was taken not to contaminate the sides of the tubes with the culture. The cultures were properly mixed and kept at room temperature. At intervals of 5, 10 and 15 minutes, 1 ml of disinfectants and phenol exposed culture was transferred from each dilution to a series of sterilized test tubes containing 5ml nutrient broth and incubated for 24 hours at 37°c.

The results were observed next day. The tubes showing turbidity were considered +ve (growth) and clear tubes as that of control as –ve (no growth). From the tubes having no growth, bactericidal and bacteriostatic action of the disinfectants at that dilution was tested by streaking on to the NA plates and incubating at 37°c for 24 hours. Growth in the plates indicated bacteriostatic action at that particular dilution and no growth showed bactericidal activity.

Calculation of Phenol coefficient

The phenol coefficient was calculated from the ratio of the greatest dilution of the disinfectant capable of killing the organisms in 10minutes but not in 5 minutes to the dilution of the phenol, killing the organisms in 10 minutes but not in 5 minutes. Thus the phenol coefficient of each organism for different disinfectant was obtained.

RESULTS AND DISCUSSION

Man has always been searching for toxic chemicals, which kills or inhibits the growth of microorganisms, mainly to prevent their infective or destructive actions. The search for these chemicals goes on continually in an effort to find the suitable one. Disinfectants are one such chemical formulation. In this present study, 4 disinfectants (lizol, phenyle, domex and stericlean) were evaluated against nine bacterial pathogens.

The disinfectant property of lizol is due to benzalkonium chloride, also known as alkyldimethyl benzyl ammonium chloride (ADBAC). ADBAC is a mixture of alkyl benzyl dimethyl ammonium chloride of various even numbered alkyl-chain lengths. The product is a nitrogenous cationic surface acting agent, belonging to the quaternary ammonium compounds. The mechanism of bactericidal or microbicidal activity is thought to be due to disruption of intermolecular interaction, that cause dissociation of cellular membrane lipid bilayer. Again solutions are bactericidal or bacteriostatic according to their concentration.

According to the present result of evaluating the disinfectant property of lizol against

nine pathogens isolated from clinical samples, it was found to be effective in different concentrations against different organisms. It was effective at the concentration of 1:1000 against *Staphylococcus aureus* ATCC 6538, a gram positive cocci and used as standard strain in 10 minute but not in 5 minutes; whereas the growth of normal clinical isolate of *Staphylococcus aureus* was inhibited at 1:800 dilution in 10 minutes.

Similarly lizol in 1:100 dilutions was effective against *Staphylococcus aureus ;*1:500 dilution of lizol was effective against *Salmonella typhi*, 1: 400 dilution was effective against *Vibro cholerae* ATCC 270, 1:100 dilution was effective against *Pseudomonas aeruginosa*, 1:900 dilution was effective against *Klebsiella pneumonia,* 1:10 against *E.coli*, 1:50 against *Proteus mirabilis and* 1: 500 against *Shigella flexneri*.

Thus, phenol coefficient of lizol for *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* ATCC 270 ,*Pseudomonas aeruginosa, Klebsiella pneumoniae, E.coli , Proteus mirabilis,and Shigella flexneri* was found to be (10.52, 8.42, 5.55 , 4.0, 0.66, 6.0, 0.11, 0.5, 5.56) respectively.

Yuce *et al*. (1996) have reported that *Pseudomonas aeruginosa* on an average 300 fold more resistant to lizol and other hypochlorite based disinfectants when present in contaminated surface than on suspension which is contradicting the present findings. This may be due to strain variation, concentration of disinfectants, environmental conditions and the methods used. The current finding is not exactly comparable with other reports due to unavailability of literature. According to Burge *et al*. (1989), quaternary ammonium compounds are considered as bound biocide. They get attached chemically to the surface providing a permanent and effective dose during sanitation (Speier and Malek 1982: Kemper & White, 1991) emphasizing the effect of lizol.

Phenol has long been used for their disinfectant, antiseptic and preservative properties. It has often been referred to as "general protoplasmic poisons". They have membrane active properties which also contribute to their overall activity. Phenyle is a disinfectant where phenol is an ingredient having microbicidal property. In the present evaluated condition phenyle was effective at a dilution of 1:100, 1:400, 1: 10, 1: 50, 1:10, 1:8, 1:4, 1: 10 and 1: 50 against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus Salmonella typhi, Vibro cholerae* ATCC 270, *Pseudomonas aeruginosa, Klebsiella pneumoniae E.coli, Proteus mirabilis* and *Shigella flexneri* and the phenol coefficient are 1.05, 4.24, 0.11, 0.5, 0.065, 0.05, 0.04, 0.1and 0.55 respectively. It showed the effectiveness of phenyle at higher concentration (1:4) for *E.coli* at 10 minutes. This revealed that *E.coli* has high resistance than all other gram –ve pathogens. The present work was also supported by previous workers in different disinfectants, where phenolic compounds were the active ingredient.

Domex is one of the commonly used household disinfectants, whose disinfectant property is due to the active ingredient sodium hypochlorite, commonly known as bleach. The action of domex on different microorganisms was presented in table 19, 20, 21, 22, 23, 24, 25, 26 and 27 respectively. Domex was effective in different dilutions i.e1:400 ,1:200 ,1:800 ,1:800 ,1:600 ,1:400 ,1:1000 , 1: 200, 1: 400 and phenol coefficient is 4.21, 2.1, 8.88, 8 , 4.0, 2.66, 10.1, 2.0 and 4.44 respectively against all the organisms tested i.e. *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* ATCC 270, *Pseudomonas aeruginosa, Klebsiella pneumoniae, E.coli, Proteus mirabilis* , and *Shigella flexneri* respectively.

Pap and co-workers (2006) reported the effectiveness of domex which is a chlorine based disinfectant against all test organisms i.e *Staphylococcus aureus, Pseudomonas aeruginosa and B. subtilis except A. niger.*

As domex is a cholorine based disinfectant, this supports the bactericidal activity of the recently studied disinfectant. Other workers like Burge *et al*. (1989) had reported the effectiveness of hypochlorite against various types of organisms demanding very short contact time.

Stericlean also contains benzalkonium chloride as an active ingredient, also known as alkyl dimethyl benzyl ammonium chloride and ADBAC is a mixture of alkyl methyl ammonium chloride of various even numbered alkyl-chain lengths. According to the present evaluation of the disinfectant property of stericlean against 9

pathogens studied. i.e. *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* ATCC 270, *Pseudomonas aeruginosa, Klebsiella pneumoniae, E.coli, Proteus mirabilis, Shigella flexneri*. Stericlean was found to be effective at a dilution of 1:1300, 1:1800, 1:1100, 1:1150, 1:1050, 1:2000, 1:1200, 1: 1800 and 1:1400, with phenol coefficient of 13.68, 18.94, 12.22, 11.5, 7.0, 13.33, 13.33, 18.0 and 15.5, respectively. Stericlean was found to be effective at lower concentration i.e 1:2000 for *Klebsiella pneumoniae*. It indicates higher sensitivity of *Klebsiella pneumoniae* for stericlean.

The present work could not be compared with the work of other workers as no such relevant literature is available on the same organism and same type of disinfectant. Other workers have reported the disinfectant property having quaternary ammonium compounds as one ingredient in their formulations.

According to Burge *et al*. (1989), quaternary ammonium compounds are considered as bound biocide. They are attached chemically to the surface providing a permanent and effective dose during sanitation emphasizing the effectiveness of stericlean.

By phenol coefficient method the disinfectant property of lizol was tested. Table-1 indicated that lizol was highly effective for only one gram positive organism tested *Staphylococcus aureus* ATCC 6538 and less effective for *Staphylococcus aureus* (clinical isolate).This indicates that the clinical isolate *S.aureus* is more pathogenic than the standard strain. Similary, out of all the gram negative organisms tested, lizol was highly effective against *Shigella flexneri* and less effective against *E.coli.*

When the effectiveness of domex against the gram positive standard strain and clinical isolate of *Staphylococcus aureus* were studied, it was found that domex was more effective towards *Staphylococcus aureus* ATCC 6538 standard strain than *Staphylococcus aureus* clinical isolate. Hence clinical isolate was more pathogenic than the standard one.

Similarly, out of all the gram negative organisms tested domex was less effective against *Proteus mirabilis* and highly effective against *Salmonella typhi.* Similarly, out of all the gram negative organisms tested phenyle was highly effective against *Shigella flexneri* and less so towards *Klebsiella pneumoniae.*

When the effectiveness of stericlean was observed for the gram positive standard strain *Staphylococcus aureus* ATCC 6538 and clinical isolate of *Staphylococcus aureus,* stericlean was found to be more effective towards *S.aureus* clinical isolate, indicating that it is less pathogenic than the standard strain.

Similarly when the gram negative organisms were compared it was observed that stericlean was more effective towards *P.mirabilis* and less effective towards *Pseudomonas aeruginosa*.

Antibiogram

The antibiotic sensitivity test indicates (Table 2 & 3) that the two gram positive organisms were studied i.e. standard strain of *Staphylococcus aureus* ATCC6538 and clinical isolate of *Staphylococcus aureus,* the standard strain was not resistant to a single antibiotic but the clinical isolate was resistant to Ampicillin (A) ,Methicillin (M) and Amoxycillin (Am) showing zone of inhibition >12mm. For gram negative organism studied, the presently used *Pseudomonas spp.* was found to be resistant against Penicillin-G (P), Vancomycin (Va), Ciprofloxacin (Cf), Ceftracidine (Ca) and thus was a multidrug resistant bacteria. Similarly *Vibrio cholerae* (ATCC270) was resistant to Penicillin–G (P), Vancomycin (Va), Ciprofloxacin (Cf) and Ceftracidine (Ce), also a multidrug resistant bacteria. *Klebsiella pneumoniae* showing resistance towards Penicillin–G (P), Vancomycin (Va), Ciprofloxacin (Cf) and Nitrofurantoin (Nf) which conclude that the *Klebsiella pneumoniae* is also a multidrug resistant bacteria.

CONCLUSIONS

A variety of commercial household disinfectants were highly effective against potential bacterial pathogens to different degree. Among all disinfectant i.e. lizol, phenyl (Black cat), domex and stericlean (\Modicare). Stericlean was found to be most effective against gram +ve strain *Staphylococcus aureus* clinical isolate and gram – ve *Proteus mirabilis* having highest phenol co efficient.

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