RESEACH ARTICLE

Evaluation of Some Common Disinfectants Against Some Gram-Negative Bacteria

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Received: 18th Dec, 19; Revised: 22th Jan, 20, Accepted: 13th Feb, 20; Available Online: 25th Mar, 2020

ABSTRACT

Seven disinfectants were used in this study, green clean, luxxtol, bittol, PAX guard, SALi, sarttol, and lenol, and their inhibitory activity was determined against nine gram-negative bacterial isolates; one isolate of each *Escherichia coli* and *Acinetobacter baumannii*, two isolates of each *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, and three isolates of *Citrobacter freundii*, by using disc diffusion method. Results demonstrated the efficacy of lenol because it was the only disinfectant that gave inhibition zones around the filter disc for all types of studied bacteria, but other disinfectants had different effects on bacterial growth.

As well as, the detection on effect of prolonged disinfectant time by exposing bacteria to disinfectant for ten minutes expecting increased efficiency of disinfectants. The results showed that bittol had the first rank in increasing its inhibition ability of bacterial growth, Green clean was the next, followed by SALi, Sartol. Lastly, Luxxtol had the last rank because it had few effects on just *A. baumannii*.

Keywords: Disinfectants, Disk diffusion method, Time factor.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.1.16

How to cite this article: Mohammad GA. Evaluation of Some Common Disinfectants Against Some Gram-Negative Bacteria. International Journal of Drug Delivery Technology. 2020; 10(1): 102-107.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The term disinfection is the use of a chemical agent—a disinfectant— or physical process to destroy pathogens but not bacterial endospores. Disinfectants are used normally only on inanimate surfaces because, at the concentrations needed to be effective, they can be toxic to living tissue. Disinfection processes can also remove the harmful products of microorganisms from materials.

The cells show marked variation in sensitivity to a given microbicidal agent. Dooms of the whole population is not simultaneous but begin when a certain threshold of the microbicidal agent (some combination of concentration and time) is met.[1]

Factors besides time govern the effectiveness of a specific agent. These factors influence the action of antibacterial agents: 1—the load of microorganisms. A higher number of contaminants requires more time to kill.[2] The nature of the microbes in the population**.** Single microbe or mixture of microorganisms.[3] The pH and temperature of the environment.[4] The concentration of the disinfectant.[5] Mode of action of the agent.[1,2]

The influence of agents is on one or more cellular targets, inflicting damage progressively until the microbe is no longer viable. Disinfectants are extensively used in health care centers like hospitals and others to control the growth of microbial cells on both inanimate surfaces and living tissues. They are aid in the prevention of nosocomial infections, and they are essential parts of infection control practices.[3] But a problem is the selection of antiseptics and agents because different bacteria vary in their response to different disinfectants.[4]

Because of the importance of disinfectant, and because of vast using them in homes, clinical institutes and other places, and necessary of the generic awareness about their, we wont to focus a light to their efficiency by the aim of this study which consists of evaluation the extent of effectiveness for some of common disinfectants available in local markets to eliminate specific pathogenic bacteria isolated from hospital environment.

MATERIALS AND METHODS

Disinfectants

Seven types of common disinfectants in local markets were chosen in the current study: Green clean (hamoudeh, jorden), Luxxtol (Luxx paris, Iraq), Bittol (taha , turkey), PAX Guard (Cyprus), SALi (Byan, Jorden), Sartol (EKAL, jorden), and Lenol (Sparten, Jorden) to detect the effectiveness of these

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disinfectants on bacteria experimentally.

Bacterial Isolates

Nine bacterial isolates were used to study its sensitivity against the disinfectants; these bacteria were isolated from the environment of hospital include intensive care unite (ICU), patient's beds, surgical tools) as mentioned in Table 1.

Culture Media

Nutrient broth and Muller-Hinton agar were used for growth activation of the bacterial isolates and evaluation of disinfectant activity on bacteria, respectively.

Disinfectant Preparation

Disinfectants were diluted according to the instruction of a manufacturing company before use.

Filter Paper Disk Method

Primary evaluation of disinfectant efficacy was performed according to the methodology described by Brown, *et al*,[5] as follows:

- Bacterial isolates were activated by culturing one pure colony in test tubes containing N. broth and incubated for 24 hours at 37° C.
- The suspension was adjusted to (almost 1x 10^8).
- Muller-Hinton agar was inoculated with 0.1 ml of bacteria and smeared uniformly by glass spreader.
- A sterile disk of filter paper was picked up and dipped it into a disinfectant. Then the disk was Placed on the cultured plate and gently pressed.
- The same procedure was repeated for each bacterial isolate.
- After incubation, the plates for 48 hours at 37°C, the zone diameters of the growth inhibition were measured and compared.

Table 1: Distribution of bacterial isolates from its sources.

	Bacterial name	*Isolate's no.*	*Source*
1-	*A. baumanii*	-*	
2-	*P.aeruginosa*	(2)	ICU
3-	*C. freundii*	(1)	
4-	*K.pneumoniae*	(2)	
5-	*P.aeruginosa*	(1)	Patient's beds
6-	*C. freundii*	(2)	
7-	*C. freundii*	(3)	
8-	*E. coli*	-*	Surgical tools
9-	*K. pneumonia*	(1)	

* : means without number because it is a single isolate.

Effect of Exposing Bacteria to Disinfectants for Ten Minutes

To gain the probabilistic benefit of the time factor, we have exposed bacterial isolates to disinfectants for ten minutes, to determine whether the survival of the bacteria with the disinfectant for this period could increase the killing intensity. So, this was achieved by:

- Each disinfectant was diluted according to the manufacturer's instruction. Disinfectants were then added to an equal amount (1:1) of bacterial culture (1x10^8 cell/mL) by mixing 0.2 mL of each in a sterile test tube.
- At zero time, 100µL of the mixture was spread on Muller-Hinton agar by using a sterile glass spreader; this was considered as a control to compare with the 10 minutes sample.
- After ten minutes, another sample (100 µL) of the mixture was spread on Muller-Hinton agar. All plates were incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

The primary detection experiment of disinfectants on bacterial isolates, as mentioned in Table 2 have shown that (lenol) is the only disinfectant that inhibited the growth of all bacterial isolates among other disinfectants. On the other hand; (Luxxtol) did not exert any inhibitory effect on any bacterial isolate used.

As for the rest of disinfectants, the degree of effect has varied on different isolates as below: The diameter of inhibition zone around the lenol disc was 21 mm for *E. coli*, Green clean disc has given 11 mm. In contrast, the inhibition zones for the Bittol, SALi, and PAX Guard were equal to 10 mm, 9 mm and 7 mm respectively, and there was no inhibition zone for Luxxtol and Sartol.

For *A. baumannii*, the Luxxtol, PAX Guard, SALi and Sartol did not demonstrate any efficacy in inhibiting this bacterium, while Green clean and Bittol had an inhibition zone equal to 8 mm. And finally, Lenol was the most effective as shown by an inhibition zone of 24 mm.

The two isolates of *K.pneumoniae* (1) and (2) have shown their complete resistance to Luxxtol, Bittol, PAX Guard and SALi, as well as, *K.pneumoniae* (1) had resistance to Green clean and Sartol. On the other hand, *K.pneumoniae* (2) has given inhibition diameter for those disinfectants of 13 mm and 8 mm respectively, Lenol has occupied the first rank in its

Table 2: Inhibition diameter (mm) for each disinfectant on bacterial spp.

C.freundii			*P.aeruginosa*		*K. pneumoniae*				
3	*2*	*1*	*2*	*1*	*2*	*1*	*A. baumannii*	*E. coli*	رمطلا
8	0	7	0	0	13	0	8	11	Green Clean
0	0	0	0	0	0	0	0	0	Luxxtol
12	0	0	0	0	0	0	8	10	Bittol
0	0	0	0	0	0	0	0	7	PAX Guard
9	0	13	0	0	0	0	0	9	SALi
10	0	0	0	0	8	0	0	0	Sartol
23	10	19	23	12	13	30	24	21	Lenol

ability to inhibit the growth for these two isolates as 30 mm and 13 mm respectively. And the value 30 mm was the highest in comparison with all isolates.

There was a unique ability for two isolates of *P. aeruginosa* regarding disinfectant resistance, except (Lenol) as mentioned above which gave an inhibition zone of 12 mm and 23 mm for *P. aeruginosa* (1) and (2).

Three isolates of *C. freundii* have shown their ability of perfect growth with the disinfectants Luxxtol and PAX Guard, while two isolates of *C. freundii* (1) and (2) had the ability to grow with Bittol and Sarttol, while isolate No. 3 of *C. fruendii* has given inhibition zone around the disks of 12 mm and 10 mm respectively.

Green clean and SALi have inhibited *C. fruendii* (1 and 2) with 7 mm and 8 mm respectively for Green disinfectant; and 13 mm and 9 mm for SALi, while *C. fruendii* had resistant to those disinfectants like *P. aeruginosa*.

Lenol remained in the first rank in its bacterial inhibitor, where the inhibition zones around disks were 19 mm, 10 mm and 23 mm for *C. fruendii* (1, 2 and 3) respectively. Photo 1 shows the ability of Lenol on *C. freundii* No. 2 growth.

The results above show that different species of bacteria differ in their response to different kinds of disinfectants.

The disk-diffusion method is utilized in laboratories to assess the efficacy of chemical agents. Filter paper disk is soaked with a chemical and put on an agar plate that has been inoculated previously and incubated with the test bacteria. After incubation, if the chemical is potent, a clear area can be seen around the disk representing inhibition of growth.[6]

Resistance to antimicrobial agents can be either a natural attribute of an organism or obtained by mutation or acquirement of plasmids or transposons. Intrinsic resistance is shown by Gram-negative bacteria. Results of this method indicate that varied pathogens acquired resistance to varied disinfectants.[7]

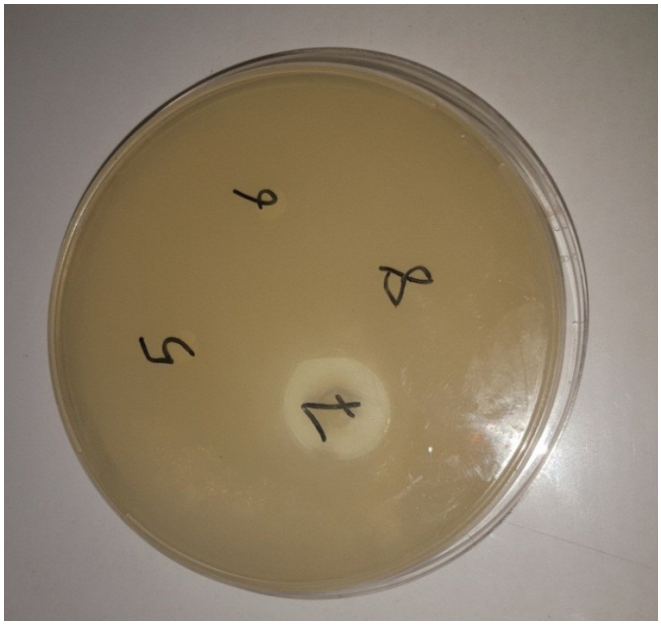

Photo 1: growth inhibition of *C. freundii* by Lenol.

Disinfectants are extensively used in hospitals and other health care centers for a variety of hard-surface applications. In particular, they are aid in the prevention of nosocomial infections, and they are a necessary part of infection control practices and Larson, E. L. *et al.* and Rutala, W.A. *et al.*[3,8]

The cellular targets of physical and chemical agents fall into four general categories: (1) cell walls, (2) cell membranes, (3) nucleic acid and protein synthesis, and (4) proteins structures and functions.[1]

Consonantal cleaning and disinfection, along with hand washing considers one the most effective measures to blocking contamination.[9]

Acinetobacter baumannii bacterium has appeared as an increasingly important pathogen worldwide. One of reports has shown that commonly used disinfectants, such as chlorhexidine, 70% ethanol, sodium hypochlorite, or quaternary ammonium compound are 100% effective against *A. baumannii* isolates when used at concentrations recommended by the manufactured company.[10]

Pseudomonas aeruginosa is an important opportunistic pathogen, and it is one of the major causes of hospital-acquired infections.[11]

Considering the evaluation of exposing bacteria to the disinfectants for ten minutes, the aim was to increase the probability of disinfectant efficacy on bacteria by the benefit of time factor, through mixing the disinfectant with bacteria with a proportion 1:1 for ten minutes to give enough time to increase the disinfectant activity, but Lenol was excluded in this experiment based on first experiment which has proved that this disinfectant was already active on bacterial isolates used. The other disinfectants were used in this experiment.

Table 3 shows the results of the disinfectant effect on bacteria after ten minutes of exposure by observing growth on Muller-Hinton agar.

This table reveals that Bittol occupied the first rank among other disinfectants in the effect of prolonged time on its activity, it has inhibited bacterial isolates of *K.pneumonia* (2), *P. aeruginosa* (2) and *C. freundii* (1).

Photo 2 shows this disinfectant ability in killing *C. freundii* after ten minutes of exposure, while growth was evident at zero time. Also, this disinfectant weakened the growth of *C.*

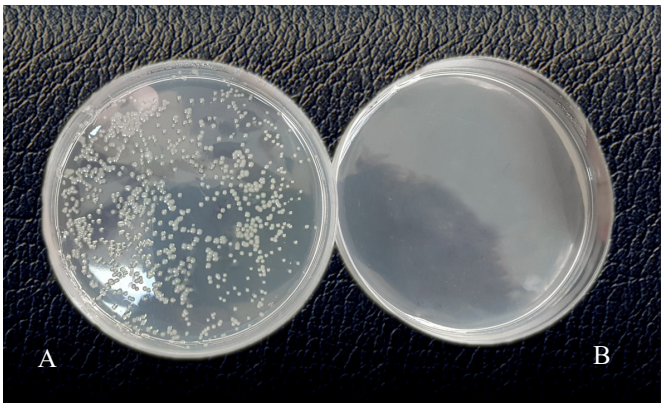

Photo 2: Growth inhibition of *C. freundii* by Bittol after ten minutes of exposing

Table 3: Effect of bacterial exposure to disinfectant for 10 minutes through bacteria growth on medium or not.

C.freundii 3		*C.freundii 2*		*C.freundii 1*		*P.aerugin-osa 2*		*P.aerugin-osa1*		*K.pneumo-niae 2*		*K.pneumo-niae 1*		*A.bauma-nnii*		*E.Coli*		*Bacteria* / *Time* / *Disinfectant*
10 min	*Zero Time*	*10 min*	*Zero Time*	*10 min*	*Zero Time*	*10 Min*	*Zero Time*	*10 min*	*Zero Time*	*10 min*	*Zero time*	*10 min*	*Zero time*	*10 min*	*Zero time*	*10 min*	*Zero time*	
NG	NG	NG	g	g	g	ND	ND	g	g	NG	NG	Wg	g	NG	NG	NG	NG	Green clear
g	g	g	g	g	g	G	g	ND	ND	g	g	ND	ND	Wg	g	ND	ND	Luxxtol
NG	NG	Wg	g	NG	g	NG	g	ND	ND	NG	g	ND	ND	NG	NG	ND	ND	Bittol
NG	NG	g	g	g	g	ND	ND	g	g	Wg	g	Wg	g	Wg	g	g	g	SAli
g	g	NG	g	g	g	G	g	g	g	g	g	NG	g	g	g	g	g	Sartol

g = Growth; NG = No growth; Wg = Weak growth ; ND = Not done

freundii after ten minutes, as well as there was no growth for *A. baumannii* and *C. freundii* for both time intervals.

Green clean ranked second, the killing effect was on *E. coli*, *A. baumannii*, *K. pneumonia* (2) and *C. freundii* (3).

Table 3 also shows the benefit of exposing time with *C. freundii* by inhibiting the growth completely after ten minutes comparing with zerotime. Green clean has weakened the growth of *K.pneumoniae* (1), while there was no noticeable effect on *P. aeruginosa* and *C. freundii* (1).

SALi ranked third among studied disinfectants, *C. freuindii* (3) did not show any resistance at both time intervals. Also, SALi weakened the growth of three bacterial isolates after ten minutes of exposure; they are *A. baumannii*, and two isolates of *K.pneumoniae*. The effect of SALi on growth of *K.pneumoniae* is shown by Photo (3), but *P. aeruginosa* (1) and *C. freundii* (1) were able to grow ordinary on culture media at both time intervals.

For Sartol, it had a positive effect in its killing two types of bacteria *K. pneumonia* (1) and *C.freundii* (2), but it has failed to kill other isolates at both time intervals.

Finally, Luxxtol had the last rank among disinfectants, through its limited ability on *A. baumannii*, and this ability was demonstrated by weak growth (not complete inhibition). Also for this disinfectant, there was no effect on the growth of other bacteria, as shown in Table 3.

Our results has agreed with[12-5] who mentioned that *P. aeruginosa* is archly resistant to most of these chemical agents, including chlorhexidine, in our study, the disinfectants: Lenol, Sarttol, and Luxxtol contain this material; also they mentioned that the outer membrane of gram-negative bacterium acts as a preventer that limits the entry of chemically unrelated types of antibacterial agents.

As well as[4,15] said that gram-negative bacteria that appear elevated levels of resistance to many disinfectants and antiseptics include *P. aeruginosa*, *Proteus* spp., *Burkholderia cepacia*, and *Providencia stuartii*. The outer membrane of bacterium *P. aeruginosa* is responsible for its high level of resistance; in comparison with other bacteria, there are varieties in LPS composition and in the cation content of the outer membrane. The elevated Mg21 content helps in producing strong LPS-LPS links; besides, because of the small size, the

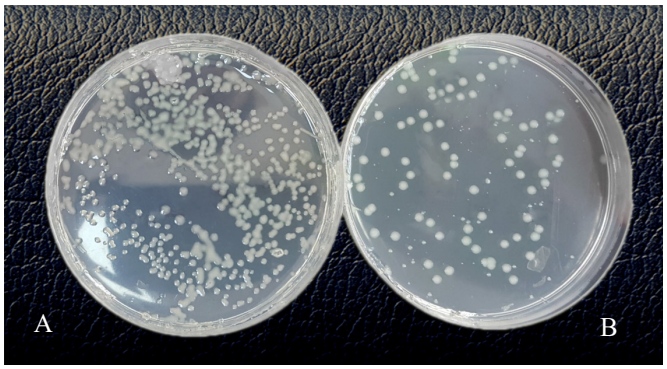

Photo 3: SALi weakened growth of *K.pneumoniae* after ten minutes of exposing; A: growth at zerotime; B: growth after ten minutes of exposure

porins perhaps not permit general diffusion through them. Also, Fentichlor affected the metabolic activities of *E. coli*. It produced a selective increase in permeability to protons with a consequent dissipation of the proton motive force (PMF) and uncoupling of oxidative phosphorylation.[16]

A. baumannii infections owe to multidrug resistance and the potency to form biofilm, which protect the bacteria from chemical agents and/or desiccation on abiotic surfaces.[10]

Lenol, PAX Guard, Sarttol and Bittol used in current study contain alcohol, it is working as antimicrobial chemical agents, Alcohol is hydrocarbons material with one or more —OH functional groups. Of many alcohols available, only isopropyl and ethyl are suitable for bacterial control. Alcohols are used as solvents for tinctures (with iodine, for example) or alone in aqueous solutions. Alcohol's mode of action depends in part upon its concentration.

A total of 50% and higher concentrations dissolve membrane lipids, disrupt cell surface tension, and compromising membrane integrity. Alcohols that have entered the protoplasm denature protein through coagulation but only in alcohol-water solutions of 50 to 95%. Alcohols are the exception to the rule that a higher concentration of an antimicrobial chemical has greater microbicidal activity. Since water is required for a protein to coagulate, alcohols show a greater bactericide activity at 70% concentration than at 100%. Absolute alcohol (100%) dissolves cell membranes and inhibits cell growth but generally is not a protein coagulant.[1]

The concentrations of disinfectants affect their action, so it must always be diluted exactly as a limitation by the manufacturer company. Also, consider the nature of the substance being disinfected. Also, the pH of the medium often has a vast effect on the activity of a disinfectant. Another very specific consideration is whether the disinfectants will easily make contact with the microbes. A surface might require to be scrubbed and rinsed before the disinfectants are applied.

Disinfection, In general, is a gradual process. So, to be effective, disinfectants might need to be left on an area for several hours.

After the end of this study, the researcher recommends using the disinfectants in homes or other places; it will be better to leave them for ten minutes or more to guarantee the best disinfection process, specially for Bittol and Sarttol disinfectant.

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مارك ةغبصل ةبلاسلا ايرتكبلا ضعب دض ةعئاشلا تارهطملا ضعب مييقت

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الملخص:

استخدمت سبعة انواع من المطهرات في هذه الدراسة وهي كل من Green Clean, Luxxtol, Bittol, PAX Guard, SALi, Sartol, Lenol وتم تعيين فعاليتها التثبيطية ضد تسع عزلات بكتيرية سالبة لصبغة كرام وهي عزلة واحدة من بكتريا *Escherichia coli* و عزلة واحدة من بكتريا *Acinetobacter baunannii* ، وعزلتين من بكتريا *Klebsiella pneumonia* ، وعزلتين من *Pseudomonas aeruginosa* وثلاث عزلات من بكتريا *Citrobacter freundii* وذلك باستخدام طريقة الانتشار بالاقراص.

وقد اظهرت النتائج كفاءة المطهر Lenol, اذ انه المطهر الوحيد الذي اعطى مناطق تثبيطية حول قرص التشريح لكل العزلات البكتيرية، وتتفاوت باقي المطهرات في تأثيرها على النمو البكتيري.

في ضوء ذلك تم التحري عن تأثير زيادة وقت التطهير وذلك من خلال تعريض البكتريا للمطهر لمدة 10 دقائق وتوقعاً الفي زيادة كفاءة المطهر. واظهرت النتائج بحالات Bittol المركز الأول في زيادة قدرته التثبيطية ونمو العزلات البكتيرية, ثم المطهر Luxxtol على المركز الأخير اذ انه لم في ذلك المطهر Grean Clean ثم المطهر SALi وجاء بعده المطهر Sarttol ، وأخيراً حصل يؤثر الا تأثيراً قليلاً على بكتريا واحدة فقط هي *A. baumannii* وذلك من خلال اضعاف نموها.