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Exploration of bactericidal effects and disinfecting agents on microorganisms

Exploración de los efectos biocidas y agentes desinfectantes en microorganismos

GONZÁLEZ– GARCÍA, Arcelia†*, REYES-ESCOBEDO, Fuensanta del Rocío, GONZÁLEZ-MARTÍNEZ, Lilia and ÁLVAREZ-GONZÁLEZ, Patricia Montserrath

Universidad Autónoma de Zacatecas, Unidad Académica de Ciencias Químicas, Unidad Académica Preparatoria, Unidad Académica de Ciencias Biológicas. México.

ID 1st Author: *Arcelia, González-García* / **ORC ID:** 0000-0003-0674-1072

ID 1st Co-author: *Fuensanta del Rocío, Reyes-Escobedo* / **ORC ID:** 0000-0002-4052-746X

ID 2nd Co-author: *Lilia, González-Martínez* / **ORC ID:** 0000-0002-3679-0070

ID 3rd Co-author: *Patricia Montserrath, Álvarez-González* / **ORC ID:** 0009-0005-0280-1025

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Abstract

Disinfection is a process aimed at the eradication of infectious microorganisms through the use of chemical or physical agents. Its function is of utmost importance in the mitigation of infectious diseases prevalent in the population, since both viruses and bacteria can subsist on inanimate surfaces, thus serving as passive vectors of pathogens. This research addresses the application of disinfectant bactericides on microorganisms such as *Staphylococcus aureus* and *Klebsiella pneumoniae*. In addition to evaluating the efficacy of disinfectants such as Zalema Naturals, Dr Beckmann, Family Guard® and Lysol®, on highly frequented surfaces, such as desks, windows, locks, sampling areas, stretchers, among others. This type of research contributes to expanding knowledge about the effectiveness of agents in preventing the spread of infectious diseases, thus strengthening hygiene and safety measures in everyday environments

Effects, Uses, Biocides, Agents, Microorganisms

Resumen

La desinfección es un proceso dirigido a la erradicación de microrganismos infecciosos mediante el empleo de agentes químicos o físicos. Su función es de suma importancia en la mitigación de enfermedades infecciosas prevalentes en la población, ya que, tanto virus como bacterias pueden subsistir en superficies inanimadas, sirviendo así como vectores pasivos de patógenos. En esta investigación se aborda la aplicación de bactericidas desinfectantes en microorganismos como *Staphylococcus aureus* y *Klebsiella pneumoniae*. Además de evaluar la eficacia de desinfectantes como Zalema Naturals, Dr Beckmann, Family Guard® y Lysol®, en superficies altamente concurridas, tales como, escritorios, ventanillas, cerraduras, áreas de toma de muestras, camillas, entre otras. Este tipo de investigación contribuye a expandir el conocimiento sobre la efectividad de agentes en la prevención de la propagación de enfermedades infecciosas, fortaleciendo así las medidas de higiene y seguridad en entornos de uso cotidiano.

Efectos, Usos, Biocidas, Agentes, Microorganismos

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* Correspondence to Author (E-mail: arcelia2009@live.com)
† Researcher contributing as first author.

Introduction

Disinfection is a process aimed at the eradication of infectious microorganisms through the use of chemical or physical agents. Its function is of utmost importance in the mitigation of infectious diseases prevalent in the population, since both viruses and bacteria can subsist on inanimate surfaces, thus serving as passive vectors of pathogens; the risk of transmission of pathogenic microorganisms increases when controls on hygiene practices are insufficient, in addition to the increase of multidrug-resistant strains in the case of bacteria (3).

Bacteria are unicellular prokaryotic microorganisms, which reproduce by binary fission. Their complex surface structure, known as the bacterial cell wall, surrounds the cell membrane, providing rigidity, and their composition confers useful and determining characteristics for taxonomy, classification and understanding of pathophysiology, as well as being the site of action of some antibiotics (1).

In the health field, it is important to note that some bacteria are involved in foodborne diseases and can persist on inert food contact surfaces, such as *Bacillus*, *Staphylococcus*, *Streptococcus*, *Pseudomonas* and *Serratia* are relevant in this context. To combat them, biocides are used, substances that, thanks to knowledge of their physical, chemical or biological properties, can neutralise, control and/or reduce the pathogenic bacterial load (5).

The action of biocides on the bacterial cell can lead to changes in cellular targets by altering them chemically, inducing mutations or creating enzymatic modifications. This includes damage to metabolic enzymes essential to the bacterium and modification of the natural permeability of the outer membrane. In addition, biocides can affect the size or number of porins, as well as cause active excretion of accumulated metabolites from the cell via the proteins that make up the efflux systems (4).

Since the 1950s, bacterial strains capable of acquiring resistance to biocides through different mechanisms have been identified, either intrinsically or through mutation, plasmid or transposon acquisition. When a lethal agent, whether physical or chemical, is exposed to a bacterial population, a progressive reduction in the number of micro-organisms is observed, and if plotted on a graph, this phenomenon follows an exponential decreasing trend over time. In this context, an ideal disinfectant should possess attributes such as being a broad-spectrum germicide, being low cost, offering broad action, being readily available, not generating subsistence and lacking an unpleasant odour (2).

Methodology

The study was a descriptive cross-sectional longitudinal study. Highly frequented surfaces were monitored, such as desks, windows, locks, sampling areas, stretchers, among others, from which 60 random samples were obtained. The samples were cultured using the plaque casting method, under optimal conditions for the adequate development of microorganisms for 48 hours, from which samples were obtained at different times, as this varied according to the disinfectant that had been applied. The exposure time was as follows: Zalema Naturals was left to act for approximately 30 seconds, Family Guard® for 2 to 3 minutes, Lysol® for 5 minutes and Dr Beckmann disinfectant for 60 seconds.

Finally, sensitivity tests were carried out using the Kirby Bauer method with gram-positive and gram-negative organisms obtained from clinical isolates donated by the Autonomous University of Zacatecas, such as Staphylococcus aureus y Klebsiella pneumonia; These were incubated and checked after 24 hours, measuring inhibition halos with a vernier. In addition, for the samples cultured with the evaluation of the disinfectants with the pour-plate method, after 48 hours of incubation, the number of microorganisms was counted developed with the help of a colony counter.

Using the statistical programme GraphPad PRISM® ver. 8.0.1 and Excel spreadsheets, data analysis was carried out, taking into account the variables studied:

- Type of disinfectant.
- Efficacy of the disinfectant.

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Results

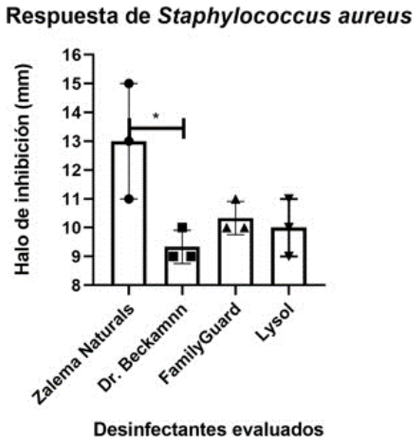
The Kirby Bauer technique was carried out in order to determine the inhibition halo size. (mm) in those that presented bactericidal activity (positive control). To interpret the inhibition halo diameters, the cut-off point tables of the commercial kit were taken into account, which take as a reference the values established in guidelines such as Clinical and Laboratory Standards (CLSI), Comnité de l'antibiogramme, French Society of Microbiology (CA-SFM) and European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Firstly, the response of Staphylococcus aureus to the disinfectants was observed in 3 monitoring tests (Table 1).

Respuesta de *Staphylococcus aureus* frente a los desinfectantes Medición del halo de inhibición (mm)				
	1	**2**	**3**	**Promedio**
Zalema Naturals	13	11	15	13
Dr. Beckmann	9	9	10	9.33333333
FamilyGuard	10	10	11	10.3333333
Lysol	10	9	11	10

Table 1 Response of *Staphylococcus aureus* to disinfectants by sensitivity test

According to the results obtained in the statistical programme GraphPad PRISM® ver. 8.0.1 (Graphic 1), a greater response was obtained for the disinfectant Zalema Naturals in comparison with the rest of the disinfectants, followed by the disinfectants Family Guard®, Lysol®, showing a lower response for the disinfectant Dr Beckmann® and Lysol®.



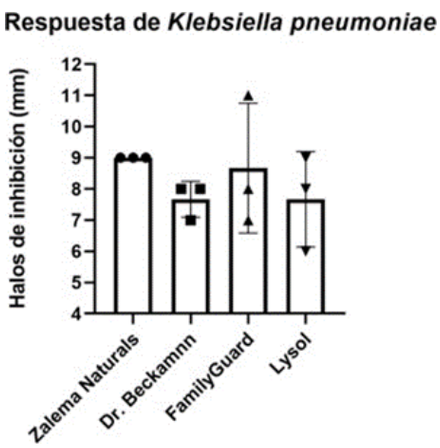
Graphic 1 Box plot for comparison of means of inhibition halos of the disinfectants evaluated against Staphylococcus aureus. Carried out in GraphPad PRISM® statistical software ver. 8.0.1
*Significant difference

On the other hand, with respect to *Klebsiella pneumoniae*, there was no difference in sensitivity due to the composition of the disinfectants tested against the strain (table 2).

Respuesta de *Klebsiella pneumoniae* frente a los desinfectantes Medición del halo de inhibición (mm)				
	1	**2**	**3**	**Promedio**
Zalema Naturals	9	9	9	9
Dr. Beckmann	7	8	8	7.66666667
FamilyGuard	7	11	8	8.66666667
Lysol	6	8	9	7.66666667

Table 2 Sensitivity test of Klebsiella pneumoniae to disinfectants tested

A higher response was shown for the disinfectant Zalema Naturals after Family Guard® and the disinfectants Dr. Beckamnn and Lysol® with the same average inhibition (Figure 2).



Graphic 2 Box plot for comparison of means of inhibition halos of the disinfectants evaluated against *Klebsiella pneumoniae*

Disinfectants evaluated against *Klebsiella pneumoniae*. Carried out in the statistical programme GraphPad PRISM®ver. 8.0.1

On the other hand, when evaluating the antimicrobial efficacy of disinfectant agents on inert surfaces, the following was observed:

The disinfectant Family Guard (quaternary ammonium derivative) presents an adequate effectiveness (Table 3), oscillating with a final average of 95.2%, presenting a greater response against *Staphylococcus aures* compared to *Klebsiella pneumoniae*.

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Dilución evaluada y desinfectante estudiado		Antes de desinfección UFC/100cm²	Después de desinfección UFC/100cm²	% de disminución población microbiana
FAMILY GUARD	1:10	10	<1	90%
	1:10	80	10	87.5%
	1:10	120	<1	99.16%
	1:100	<1	8,300	CONTAMINADA
	1:100	300	<1	99.66%
	1:100	500	<1	99.8%
	1:1000	<1	5,000	CONTAMINADA
	1:1000	<1	<1	SIN CAMBIOS
	1:1000	<1	<1	SIN CAMBIOS

Table 3 Effectiveness of Family Guard® disinfectant

In addition, it was observed that Zalema Naturals (derived from citrus extracts) has a higher bactericidal effectiveness than all the tested products applied in inert material, both in the evaluation of sensitivity against *Staphylococcus aureus* and *Klebsiella pneumoniae* strains, and in the evaluation of efficacy, with a final average ranging between 96% disinfection (Table 4).

Dilución evaluada y desinfectante estudiado		Antes de desinfección UFC/100cm²	Después de desinfección UFC/100cm²	% de disminución población microbiana
ZALEMA NATURALS	1:10	350	<1	99.71%
	1:10	<1	<1	SIN CAMBIOS
	1:10	10	<1	90%
	1:100	10	<1	90%
	1:100	100	<1	99%
	1:100	0	CONTAMINADA	CONTAMINADA
	1:1000	9,000	1,000	99.99%
	1:1000	<1	<1	SIN CAMBIOS
	1:1000	1,000	<1	99.9%

Table 4 Effectiveness expressed as a percentage of the disinfectant Zalema Naturals applied on inert matter

Likewise, the disinfectant evaluated, Lysol, shows some of its monitoring with a low percentage of reduction, thus calling into question an effective disinfection, however, it has favourable reduction percentages (Table 5). However, it has favourable reduction percentages (Table 5).

Dilución evaluada y desinfectante estudiado		Antes de desinfección UFC/100cm²	Después de desinfección UFC/100cm²	% de disminución población microbiana
LYSOL	1:10	<1	20	CONTAMINADA
	1:10	30	10	66.66%
	1:10	90	<1	98.88%
	1:100	600	<1	99.83%
	1:100	<1	<1	SIN CAMBIOS
	1:100	300	100	66.66%
	1:1000	3,000	<1	99.66%
	1:1000	<1	<1	SIN CAMBIOS
	1:1000	<1	<1	SIN CAMBIOS

Table 5 Effectiveness expressed as a percentage of the disinfectant Lysol®

On the other hand, the disinfectant Dr. Beckmann (quaternary ammonium derivative) showed a lower effectiveness compared to those tested, with an average effectiveness of 81.9% (Table 6).

Dilución evaluada y desinfectante estudiado		Antes de desinfección UFC/100cm2	Después de desinfección UFC/100cm2	% de disminución población microbiana
DR. BECKMANN	1:10	1,490	10	99.3%
	1:10	10	10	0%
	1:10	30	<1	96.66%
	1:100	6,900	<1	99.99%
	1:100	<1	<1	SIN CAMBIOS
	1:100	1,800	5,900	CONTAMINADA
	1:1000	9,000	<1	99.98%
	1:1000	<1	<1	SIN CAMBIOS
	1:1000	23,000	1,000	95.65%

Table 6 Effectiveness expressed as a percentage of Dr. Beckmann disinfectant

To conclude, one of the most important variables to consider when making a purchasing decision is the relationship between the cost and the benefit to be obtained (Graph 3). The calculations in relation to the cost were carried out with the price established in the months of September to December 2021.

From the above, it can be concluded that the surface disinfectant Family Guard is one of the most economical, followed by the disinfectant Lysol, concluding that the disinfectant Dr. Beckmann has a high cost due to its smaller presentation.

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	FamilyGuard 650mL	Lysol 650mL	Zalema Naturals 100mL	Dr Beckmann 50mL
Precio modificado a 100mL	$8.61	$10.10	$25	$63.20
Precio modificado a 650mL	$56	$65.70	$162.50	$410.80
Precio establecido	$56	$65.70	$25	$31.60

Graphic 3 Cost-benefit ratio for each of the disinfectants evaluated. Carried out in the Excel statistical programme

The analysis of the results corroborates the antimicrobial capacity of the agents evaluated in this research, as well as the comparison of each one in relation to their effectiveness.

Conclusions

From the results obtained, we can observe that, despite the fact that disinfection was not carried out using the classic method, i.e. prior cleaning (water and soap/detergent) and the microbicide agent, with the sole application of the disinfectant agent, more than 80% of the microorganisms on the surface were eliminated in most cases. This is of utmost relevance as it helps to mitigate infectious diseases prevalent in the population, protecting public health.

References

1. Álvarez, O. (2016). La membrana plasmática y la pared celular. Citosol y citoesqueleto. Sistemas de membranas y orgánulos y motilidad celular. Publicaciones Didacticas. No 73: 68-174

2. Cabrera, C., Gómez, R., & Zúñiga, A. (2007). La resistencia de bacterias a antibióticos, antisépticos y desinfectantes una manifestación de los mecanismos de supervivencia y adaptación. Colomb Med. 38(2): 149-158.

3. Diomedi, A., Chacón, E., Delpiano, L., Hervé, B., Jemenao, M., Medel, M., Quintanilla, M., Riedel. H., Tinoco, J. & Cifuentes, M., (2017). Antisépticos y desinfectantes: apuntando al uso racional. Recomendaciones del Comité Consultivo de Infecciones Asociadas a la Atención de Salud, Sociedad Chilena de Infecto logia. Rev. Chilena Infectol. 34(2):156-174.

4. Escobedo, A., Menseses, M. & Castro, A. (2016). Estudio microbiológico (cualitativo y cuantitativo) de superficies inertes que están en contacto con la preparación de alimentos en cafeterías de una universidad pública. Revista Electrónica sobre Cuerpos Académicos y Grupos de Investigación en Iberoamérica. 36(6) ISSN: 2448-6280.

5. Fajardo, L. (2021). Evaluación de desinfectantes para uso en el entorno farmacéutico. Ars Pharmaceutica. 62(2):175-18].

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