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Efecto residual de desinfectantes de uso hospitalario frente a *Acinetobacter baumannii*

Residual Effect of Hospital-Use Disinfectants against Acinetobacter baumannii

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Palabras claves: Acinetobacter baumannii, efecto residual, desinfectante, infección nosocomial, prevención, microbiología.

Resumen

Introducción. *Acinetobacter baumannii (A. baumannii)* es un patógeno multirresistente responsable de infecciones nosocomiales principalmente en la unidad de cuidados intensivos (UCI) y en pacientes inmunocomprometidos. Como medida para evitar la propagación de la bacteria, es necesario realizar la desinfección frecuente en las áreas de atención al paciente y los instrumentos empleados para ello. Por lo que resulta de gran importancia evaluar el efecto residual de los desinfectantes recomendados por el Ministerio de Salud Pública para ser utilizados en ambientes hospitalarios. **Objetivo.** Verificar la efectividad residual de yodopovidona, peróxido de hidrógeno, glutaraldehído, clorhexidina, hipoclorito de sodio, amonio cuaternario y monopersulfato de potasio empleados como desinfectantes de uso hospitalario frente a *A. baumannii*. **Metodología.** Se trató de un estudio cuantitativo, descriptivo y longitudinal, se emplearon cepa de *A. baumannii,* sobre las cuales se evaluó el efecto residual de clorhexidina, hipoclorito de sodio, amonio cuaternario y monopersulfato de potasio en diferentes periodos de tiempo. **Resultados.** El glutaraldehído y la clorhexidina fueron los desinfectantes con mejor efecto residual, manteniéndose efectivos hasta las 24 horas, sin embargo, sus halos de inhibición fueron de diámetro pequeño, con lo que sugiere una posible resistencia a los mismos. **Conclusión.** Los desinfectantes con mejor efecto residual sobre las *A. baumannii* fueron glutaraldehído y clorhexidina, con inhibición de la bacteria hasta las 24 horas después de su aplicación. De igual forma. El peróxido de hidrógeno obtuvo este efecto hasta las 12 horas. Las concentraciones del hipoclorito de sodio, la yodopovidona y el amonio cuaternario no mostraron efecto residual. **Área de estudio general:** Bioquímica y Farmacia. **Área de estudio específica:** Microbiología. **Tipo de estudio:** Artículo original / Original article.

Keywords: Acinetobacter baumannii, residual effect, disinfectant,

Abstract

Introduction. *Acinetobacter baumannii (A. baumannii)* is a multidrug-resistant pathogen responsible for nosocomial infections, in the intensive care unit (ICU) and

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nosocomial infection, prevention, microbiology.

immunocompromised patients. As a measure to prevent the spread of the bacteria, it is necessary to disinfect patient care areas and instruments. Therefore, evaluating the residual effect of the disinfectants recommended by the Ministry of Public Health in hospital environments is essential.**Objective.**To verify the residual efficacy of iodopovidone, hydrogen peroxide, glutaraldehyde, chlorhexidine, sodium hypochlorite, quaternary ammonium, and potassium monopersulfate used as hospital disinfectants against A. baumannii.**Methodology.**This was a quantitative, descriptive, and longitudinal study using the strain A. baumanni ATCC 19606 on which the residual effect of chlorhexidine, sodium hypochlorite, quaternary ammonium, and potassium monopersulfate was evaluated at different periods: 20 minutes, one, three, six, 12 and 24 hours.**Results.**Glutaraldehyde and chlorhexidine were the disinfectants with the best residual effect, remaining effective for up to 24 hours; However, their inhibition halos were small in diameter, suggesting a potential resistance to these disinfectants.**Conclusion.**The disinfectants with the best residual effect on A. baumannii were glutaraldehyde and chlorhexidine, inhibiting the bacteria up to 24 hours after application. Similarly, hydrogen peroxide obtained this effect up to 12 hours. The sodium hypochlorite, iodopovidone and quaternary ammonium concentrations showed no residual effect.

Introduction

The growing resistanceof*Acinetobacter baumannii,*to antibiotics and disinfectants presents an urgent challenge to public health worldwide. The Acinetobacter genus is a genus of gram-negative bacteria that are widely distributed in water, soil, and human skin. Although it has a low level of virulence, cases of infections in hospitalized patients are becoming more frequent (1). In this group of microorganisms, Acinetobacter baumannii stands out, a multi-resistant pathogen responsible for nosocomial infections mainly in the intensive care unit (ICU) and in immunocompromised patients. From a clinical point of view, it can cause prolonged hospital stays, a higher mortality rate, and broad-spectrum resistance (2).

Since 2015, the Infectious Diseases Society of America has classified A. baumannii as one of the six most clinically important bacteria worldwide due to its multi-resistance and its association with infections such as pneumonia, meningitis, bacteremia, urinary tract infections, and skin and soft tissue infections. In Latin America, it is estimated that on average 90% of nosocomial infections have been caused by A. baumannii. (3). The residual effect of hospital disinfectants refers to the ability of a product to maintain its antimicrobial action for a prolonged period after its initial application (4).

In recent years, infection by this bacteria has increased its prevalence and with it its resistance mechanisms, increasing in the United States from 4% to 9%. In Latin America, it is estimated that, on average, more than 50% of nosocomial or intrahospital infections of patients in ICU have been caused by A. baumannii, this is because it has generated different resistance mechanisms that include enzyme synthesis, modifications in membrane transport, which have limited the effectiveness of penicillins, aminoglycosides, tetracyclines and quinolones (5).

In Mexico, the frequency of nosocomial infections has been determined to range between 2.1 and 35.4% distributed across all hospital units. Although gram-positive bacteria are more frequent, gram-negative bacteria such as A. baumannii have a greater predisposition to septic shock and death. This pathogen under normal conditions can be part of the normal microbiome of the oral cavity and respiratory tract in healthy subjects; however, in immunocompromised patients it is responsible for 90% of nosocomial infections and 92% of nosocomial bacteremias (6).

Likewise, a study carried out by Encalada and Arteaga (5) in Ecuador determined that in the last 10 years the level of resistance of this bacteria has increased considerably, making its therapeutic management difficult. Currently, resistance to carbapenems, antibiotics of first choice for treatment, has increased considerably. This situation has been related to patient conditions such as age, comorbidities, as well as the misuse of protective measures by health personnel and inadequate disinfection of surfaces.

As a strategy to prevent this type of infections, biocidal substances are used, including antiseptics, disinfectants and preservatives, whose properties are not specific to a single pathogen, but rather broad spectrum, so they are not considered specific like antibiotics. Their function is to inhibit the growth of microorganisms or eliminate them for use on surfaces and objects. The main products used are: quaternary ammonium derivatives (benzalkonium chloride), biguanides (chlorhexidine), phenols (triclosan), alcohols (ethanol or ethyl alcohol), aldehydes (glutaraldehyde), halogenated compounds (iodine and chlorine) and hydrogen peroxide (7).

Sodium hypochlorite (NaOCl) is a chlorine-based disinfectant that is commonly used in different environments, including domestic and hospital settings. The health system of

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each country has stipulated different chemical compounds and the appropriate concentration for their use, because when used inappropriately it can cause adverse health effects or resistance to them (8).

Hypochlorite is one of the most widely used disinfectants because it is highly available and its effectiveness in inhibiting different microorganisms has been proven, as well as generating residual effects. It is even used in the treatment of wastewater from health centers. The effectiveness of this product against pathogens depends on factors such as the concentration used and the exposure time (9).

In relation to potassium monopersulfate, it is part of the peroxygen compounds, and can also be known by other names such as potassium persulfate, among others. Its mechanism of action consists of promoting the oxidation of bacterial structures, generating the death of the microorganism. According to the classification of the Centers for Disease Control and Prevention (CDC), it is a broad-spectrum disinfectant with an intermediate level of effectiveness against fungi, bacteria and certain viruses (10).

Hydrogen peroxide, also known as hydrogen peroxide, is a very stable chemical substance that must be kept in places that prevent the passage of light and it is recommended to use opaque containers for this purpose. It is characterized by presenting bactericidal, bacteriostatic or sporicidal action. According to the concentrations, it has been determined that 3% is bacteriostatic and 6% is bactericidal. It does not have a prolonged effect and its mechanism of action is based on the production of hydroxyl ions and free radicals, which generate the oxidation of essential compounds for pathogens such as lipids, proteins and DNA. The elimination of spores occurs through the release of O2 preventing them from germinating (11).

Another frequently used disinfectant in hospital environments is quaternary ammonium, which is characterized by being a surfactant, bactericide and has an inhibitory effect on viral activity. Its effectiveness is due to its broad spectrum, which includes gram-positive and gram-negative bacteria, fungi and viruses such as hepatitis B and HIV (12).

In relation to glutaraldehyde, it is a liquid, oily, colorless substance with a sharp odor, also known as pentanedial, glutaral, 1,5-pentanedial. Because it is not stable in its chemical form, it must be diluted in water. It is frequently used in different environments, including hospitals, as a disinfectant for surfaces and equipment (13). The effectiveness of these substances depends on the mechanism of action of their active ingredients, as well as the residual effect that they are able to generate. This is defined as one of the greatest properties of disinfectants, consisting of being able to maintain bacterial inhibition for several hours (14).

Thus, the prevention of nosocomial infections caused by this pathogen has become a priority for health systems. One of the biosecurity measures that can be used is the use of chemical substances to disinfect surfaces that are in contact with the patient. The effectiveness of these depends on their concentration and mechanism of action. Due to the clinical importance of A. baumanni, the present investigation aimed to verify the residual effectiveness of povidone-iodine, hydrogen peroxide, glutaraldehyde, chlorhexidine, sodium hypochlorite, quaternary ammonium and potassium monopersulfate used as hospital disinfectants against A. baumannii.

Methodology

The study was developed using a non-experimental design. The work allows to verify the residual effect of the disinfectants mentioned in the biosafety manual of the Ministry of Public Health (MSP) for use in hospital environments in different time periods: 20 minutes, 1 hour, 3 hours, 6 hours, 12 hours and 24 hours.The sample consisted of six disinfectants frequently used in hospital environments according to the concentrations established by the MSP: quaternary ammonium 0.1%, chlorhexidine 2%, sodium hypochlorite 0.5%, glutaraldehyde 2%, potassium monopersulfate 1%, povidone iodine 10% and hydrogen peroxide 6%.

The residual effect was evaluated on the A. baumannii ATCC 19606 strain. For sample selection, the following factors had to be considered:Disinfectants most commonly used in hospitalsin the concentrations proposed in the MSP or WHO biosafety manual.Disinfectants that are not widely used in hospitals or concentrations that are not proposed by the MSP or WHO were not considered.Repetitions were performed for each established time to measure the residual effect at 20 minutes, 1 hour, 3 hours, 6 hours, 12 hours and 24 hours.

For laboratory analysis, the strain of*A. baumannii*ATCC 19606, because it is resistant to disinfectants used in hospital environments.This strain was activated on McConkey agar, in additionFive repetitions were carried out per disinfectant to verify greater accuracy in the results. by exhaustion sowing at 37° for 24 hours prior to analysis. Disinfectants were prepared by diluting them in glass containers with water, and the concentrations were adjusted according to the MSP. 500 ml solutions were prepared, the concentrations of which are indicated in the following table:

Table 1.*Concentrations used for disinfectants*

Disinfectant	Concentration
Sodium hypochlorite	0.5%
Quaternary ammonium	0.1%
Chlorhexidine	2%
Glutaraldehyde	2%

Potassium monopersulfate	1%
Povidone iodine	10%
Hydrogen peroxide	6%

According to the technique of Bernal et al. (15) To verify the residual effect of disinfectants and antiseptics, previously prepared disinfectants were impregnated on filter paper discs and the Kirby Bauer antimicrobial susceptibility testing disc diffusion technique was used. The concentration of A. baumannii was adjusted using a bacterial suspension up to 0.5 according to the McFarland scale, which corresponds to 1.5 x 108 CFU/ml. For this purpose, 0.9% saline solution was used until the necessary turbidity was reached by reading absorbance between 0.08 and 0.12 in the spectrophotometer at a wavelength of 600 nm.

The residual effect test was performed on Muller Hilton agar and the mass plating technique was used to ensure uniform bacterial growth throughout the plate with a sterile swab. The disinfectant susceptibility test was performed by placing the previously disinfectant-impregnated discs (Table 1) on the bacterial cultures on Mueller Hinton agar at different time periods.

For the analysis of the residual effect of antiseptics and disinfectants, the Duraffourd classification was used, a scale that classifies the sensitivity values of antimicrobial substances, allowing to define whether the pathogen is resistant or not according to the diameter of the inhibition zone. Table 2 details the classification based on the inhibition zones (16):

Table 2.*Duraffourd classification for inhibition halos*

Status	Reference value for inhibition halos
Null (-)	≤ 8 mm
Sensitive (+)	> 9 mm ≤ 14 mm
Very Sensitive (++)	>15mm ≤ 19mm
Extremely sensitive (+++)	> 20 mm

Fountain: Morillo & Balseca (16)

Results

The results obtained from the measurement of the inhibition halos of the disinfectants during the research times are detailed in Table No. 3.

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Table 3. *Average of inhibition halos*

Time	Average inhibition halos (mm)					
	20 min	1 hour	3 hours	6 hours	12 hours	24 hours
Sodium hypochlorite	7.4	0.0	0.0	0.0	0.0	0.0
Potassium monopersulfate	0.0	0.0	0.0	0.0	0.0	0.0
Hydrogen peroxide	11.4	8.9	8.6	7.4	6.1	0.0
Glutaraldehyde	9.4	8.6	8.0	7.8	7.6	6.8
Chlorhexidine	12.0	9.6	8.4	8.0	7.2	7.0
Povidone iodine	7.5	7.4	7.2	7.0	6.1	6.0
Quaternary ammonium	8.8	7.8	7.4	7.0	6.8	6.3

As detailed, sodium hypochlorite and potassium monopersulfate did not present a residual effect, due to the absence of inhibition halos by the bacteria, which leads to the conclusion that A. baumannii is resistant to the residual effect of both disinfectants and their concentrations. In the case of hypochlorite, an inhibition halo of 7.4 mm is observed after 20 minutes. However, according to the Duraffourd scale, the size of the inhibition halos demonstrates resistance by A. baumannii. In the case of potassium monopersulfate, the absence of inhibition halos was observed throughout all time periods, therefore, no residual effect is observed.

In the case of hydrogen peroxide or peroxidehydrogen, the formation of inhibition halos was observed up to three hours after its application. Similarly, when comparing with the scale ofDuraffourdIt can be observed that the diameters of the halos obtained in the first three time periods (20 minutes, 1 hour and 3 hours) allow them to be classified as sensitive. However, their residual effect is reduced over time, since it is observed that the diameter of the inhibition halo turns out to be smaller at the end than at 20 minutes of application, until it is considered resistant according to the scale.

As for glutaraldehyde, inhibition halos by A. baumannii are observed in all the repetitions, as with the effect produced by hydrogen peroxide. The comparison of the diametersobtained with the scale ofDuraffourd determined that the bacteria was resistant in cultures of 6 hours, 12 hours and 24 hours, as they presented inhibition halos with diameters less than 8.00 mm. For the times of 20 minutes, 1 hour and 3 hours, halos were obtained that decreased in the size of their halos, which according to the scale correspond to the sensitive category since the diameter was greater than 9 mm. This indicates that the residual effect was only present in the three time periods.

In the case of povidone-iodine, inhibition halos are also observed, however, itsThe diameter is small and therefore it is considered that the bacteria is not sensitive to it, since values less than 9 mm were obtained in all time periods. A similar behavior is observed with quaternary ammonium since the formation of inhibition halos of size less than 9 mm is evident except at 20 minutes of application.

Finally, almost all the products used in research with disinfectant or antiseptic effects can be deduced to have a residual effect due to the formation of inhibition halos, which decrease over time, but are not sufficient to eliminate the microorganism due to its resistance. The only disinfectant that did not generate any inhibition halo was potassium monopersulfate, a product used in low-level disinfection processes.

Discussion

Disinfectants are substances necessary to prevent infections by intra-hospital pathogens and to limit the number of nosocomial infections. According to Tyski et al. (17), the use of these substances with a bactericidal effect in standardized concentrations helps to considerably reduce the number of infectious diseases caused by microorganisms of high clinical importance. The research worked with the concentrations established by the MSP or WHO, which should guarantee the elimination of a high percentage of these on inert surfaces, even when they are not used at a hospital level.

However, research on the residual effectiveness of disinfectants does not have the necessary information, mainly on A. baumannii. In this regard, Flores (18) points out that the use of disinfectants and antiseptics are essential for the control of nosocomial infections, therefore, substances such as chlorhexidine, povidone-iodine, alcohol and alcohol gel used as antiseptics independently show a similar bactericidal effect, however, only chlorhexidine has a residual effect against A. baumannii.

Based on the above, it should be noted that the concentrations in which antiseptics or disinfectants are used are not always the indicated ones, for this reason it is necessary to know the most suitable concentration and dilution to achieve inhibition of bacterial growth without affecting the health of users. It should also be considered that pathogens develop resistance mechanisms in such a way that the concentrations of disinfectants become ineffective. This problem is further aggravated by the residual effect that they must have.

In the present study, the residual effect of several disinfectants was evaluated by means of an antimicrobial susceptibility test, among which it is mentioned that chlorhexidine presented the greatest inhibitory power present from 20 minutes to 24 hours, with halos of up to 12.4 mm. In the research by Aguiar et al. (19) they evaluated the effect of the same chemical compound with the same concentration on different cultures of A. baumanni. The results determined that the bacteria was sensitive to the compound, including multi-resistant strains. The findings found by the authors allowed them to determine that the disinfectant is effective as a measure to prevent nosocomial infections caused by this bacteria.

For his part, Bravo (20) in his research verifies the viability and culturability of A. baumannii against sodium hypochlorite with a concentration of 0.5% and hydrogen peroxide at 2%. He demonstrated that the two disinfectants are effective in a short period of time, in concentrations used at hospital level, however, they do not have a great effect on the bacteria. Of the two disinfectants used, hypochlorite is more effective compared to hydrogen peroxide, which is why the use of sodium hypochlorite is recommended more frequently. Although it is true, this research does not present the same methodology and techniques as our study, but its data reflect the importance of hypochlorite and hydrogen peroxide with bacteriostatic effect, mainly with sodium hypochlorite.

However, it is important to note that certain limitations were found in the development of the study, such as the lack of comparison between the concentrations indicated by the MSP and the WHO and others adapted by the researcher. In addition, possible combinations between disinfectants that could create synergy to intensify their mechanism of action were not evaluated. However, the completion of the research provides an updated overview of the reality of A. baumannii resistance to commonly used disinfectants.

According to Chacón and Rojas (21), studying the effect of disinfectants on bacteria of clinical interest is a very useful tool to deduce the potential for antimicrobial resistance of pathogens. In their study, they determined that there is a direct relationship between resistance to disinfectants used in hospitals and the development of resistance to antibiotics. This situation was also observed in the present study. A. baumannii is a pathogen that has developed multi-resistance to both antibiotics and most disinfectants used in hospitals at the concentrations recommended by regulatory bodies.

In this regard, Monsalve and Moscoso (22) pointed out that disinfectants that were previously effective against most of the bacteria found in hospitals have now developed resistance mechanisms against disinfectants and antiseptics such as quaternary ammonium and halogenated products. This situation has led to the isolation of bacteria resistant to disinfectant substances even in domestic and other environments, a problem that continues to worsen continuously.

Conclusions

- The research verified the residual effect of frequently used disinfectants against A. baumannii, a multi-resistant bacteria considered to cause nosocomial infections. To do so, the chemical compounds were used in the concentrations indicated by the MSP and the WHO. It was shown that the disinfectants that had a residual effect on the strain were glutaraldehyde, chlorhexidine, povidone-iodine and quaternary ammonium.Only the four disinfectants maintain a residual effect of 24 hours, however, according to the Duraffourd scale, Although there is a residual effect through the formation of inhibition halos, this is not sufficient to develop sensitivity and therefore inhibit the bacteria. The same occurs with sodium hypochlorite, although the formation of inhibition halos is observed in the first 20 minutes, this is low.

- This study compared the residual effect of different disinfectants used against A. baumannii. It was observed that four disinfectants showed a residual effect in 24 hours, which were glutaraldehyde, chlorhexidine, povidone-iodine and quaternary ammonium followed by hydrogen peroxide after up to 12 hours and finally sodium hypochlorite after only 20 minutes. However, potassium monopersulfate did not show any greater benefit. Therefore, it was concluded that glutaraldehyde, chlorhexidine, povidone-iodine and quaternary ammonium demonstrated great effectiveness, allowing continuous protection against A. baumannii.

Recommendations

With the results obtained, it is recommended to continue with more studies that analyze an increase in the concentrations of the same disinfectants or antiseptics, as long as these do not cause possible damage to the personnel who handle them, in order to identify which is the most suitable product to prevent the indirect transmission of microorganisms through inert spaces at the hospital level mainly. Additionally,HE suggests continuing with research that evaluates new products that allow the combination of disinfectants according to the surfaces to be disinfected, considering the compositions and chemical compatibility or continuing the search for new products with biocidal or bactericidal properties that prevent the spread of these.

Conflict of interest

Authors must declare whether or not there is a conflict of interest in relation to the submitted article.

Authors' contribution statement

The article must be accompanied by a note, which expresses the contribution of each author to the study carried out.

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