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Comparative analysis of the antimicrobial activity of peracetic acid and sodium hypoclorite in the fight against *Staphylococcus aureus*

Análise comparativa da atividade antimicrobiana do ácido peracético e hipoclorito de sódio no combate a *Staphylococcus aureus*

Análisis comparativo de la actividad antimicrobiana del ácido peracético y el hipoclorito de sodio para combatir *Staphylococcus aureus*

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Jorddy Neves da Cruz
ORCID: https://orcid.org/0000-0003-0529-3714
Universidade Federal do Pará, Brazil
E-mail: jorddynevescruz@gmail.com
Luciano Augusto de Sousa
ORCID: https://orcid.org/0000-0002-7931-8307
Secretaria de Estado de Educação, Brazil
E-mail: professorlucianoaugust@gmail.com
Paulo Cairo Nunes de Oliveira
ORCID: https://orcid.org/0000-0001-6856-0934
Escola Superior da Amazônia, Brazil
E-mail: paulocairo2017@outlook.com
Marcio Marcelo da Silva Pessoa
ORCID: https://orcid.org/0000-0003-4804-9690
Universidade Federal do Pará, Brazil
E-mail: mmpessoa@icloud.com
Nathiel Sarges Moraes
ORCID: https://orcid.org/0000-0002-4727-0951
Universidade Federal do Pará, Brazil
E-mail: nathiel.sed1@gmail.com
Maria Jaqueline Bailão Silva
ORCID: https://orcid.org/0000-0003-1593-1118
Universidade Federal do Pará, Brazil
E-mail: jaquebio.js@gmail.com
Ayrton Barradas Rocha
ORCID: https://orcid.org/0000-0001-5518-7616
Faculdade Integrada Brasil Amazônia, Brazil
E-mail: rocha.crbm3565@gmail.com
Fernando Costa
ORCID: https://orcid.org/0000-0003-2981-4246
Universidade Federal do Pará, Brazil
E-mail: mcosta.fernando@gmail.com
Davi do Socorro Barros Brasil
ORCID: https://orcid.org/0000-0002-1461-7306
Universidade Federal do Pará, Brazil
E-mail: davibb@ufpa.br
Stanley Soares Xavier
ORCID: https://orcid.org/0000-0002-4040-4144
Universidade do Estado do Pará, Brazil
E-mail: stx.xavier@yahoo.com.br
José de Arimatéia Rodrigues do Rego
ORCID: https://orcid.org/0000-0003-0891-6438
Universidade Federal do Pará, Brazil
E-mail: jr2rego@gmail.com

Abstract
Analyze compared to antimicrobial activity of peracetic acid and sodium hypochlorite against *S. aureus*, showing the best reagent in the inhibition of the same, in addition to quantify the concentration minimum required to inhibition growth. methodology: for the experimental group I (peracetic acid), were used 10 microtubes getting 900µl reagent and dilutions serial of 10-1 to 10-9, for the experimental group II (sodium hypochlorite) another 10 microtubes received 900µl reagent and dilutions serial of 10-1 to 10-9. It was added more 100µl the inoculum bacterial to

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microtubes of both groups, the mixture was incubated greenhouse bacteriological for 5 minutes at 37ºc and later sown in culture medium. The peracetic acid showed efficiency inhibition of growth of *S. aureus* to the dilution of 10-2, this being dilution the 0,001% of peracetic acid. In dilution of 10-3 there was a growth part of 44% compared to the control group, in dilutions later there was 100% bacterial growth. sodium hypochlorite showed effectiveness to the dilution of 10-4 being this dilution corresponding to the sodium hypochlorite the 0,0001%. In dilution of 10-5 sodium hypochlorite had an effect partial 86% of bacterial growth in relation to the control group, in dilutions later there was 100% bacterial growth. The Sodium hypochlorite showed be ten times more effective than the peracetic acid in relation to inhibition of growth of *Staphylococcus aureus.*
Keywords: Peracetic acid; Sodium hypochlorite; *Staphylococcus aureus.*

Resumo

Analisamos comparativamente a atividade antimicrobiana do ácido peracético e hipoclorito de sódio contra o *S. aureus*, evidenciando o melhor reagente na inibição do mesmo, além de quantificar a concentração mínima necessária para a inibição de crescimento. Para o grupo experimental I (ácido peracético), foram utilizados 10 microtubos recebendo 900µL do reagente e diluições seriadas de 10^{-1} a 10^{-9}, para o grupo experimental II (hipoclorito de sódio) outros 10 microtubos receberam 900µL do reagente e diluições seriadas de 10^{-1} a 10^{-9}. Foi acrescentado mais 100µL do inóculo bacteriano aos microtubos de ambos os grupos, a mistura foi incubada em estufa bacteriológica por 5 minutos à 37ºC e posteriormente semeadas em meio de cultura. O ácido peracético mostrou eficiência na inibição de crescimento do *S. aureus* até a diluição de 10^{-2}, sendo esta diluição a 0,001% do ácido peracético. Na diluição de 10^{-3} houve um crescimento parcial de 44% em relação ao grupo controle, nas diluições posteriores houve 100% de crescimento bacteriano. O hipoclorito de sódio mostrou eficácia até a diluição de 10^{-4} sendo essa diluição correspondente ao hipoclorito de sódio a 0,0001%. Na diluição de 10^{-5} o hipoclorito de sódio teve um efeito parcial de 86% de crescimento bacteriano em relação ao grupo controle, nas diluições posteriores houve 100% crescimento bacteriano. O hipoclorito de sódio mostrou ser dez vezes mais eficaz que o ácido peracético em relação a inibição de crescimento do *Staphylococcus aureus.*
Palavras-chave: Ácido peracético; Hipoclorito de sódio; *Staphylococcus aureus.*

Resumen

Analizamos comparativamente la actividad antimicrobiana del ácido peracético y el hipoclorito de sodio frente a *S. aureus*, mostrando el mejor reactivo para su inhibición, además de cuantificar la concentración mínima necesaria para la inhibición del crecimiento. Para el grupo experimental I (ácido peracético) se utilizaron 10 microtubos recibiendo 900µL del reactivo y diluciones seriadas de 10-1 a 10-9, para el grupo experimental II (hipoclorito de sodio) otros 10 microtubos recibieron 900µL del reactivo y diluciones seriadas diluciones de 10-1 a 10-9. A los microtubos de ambos grupos se les añadió 100µL adicionales de inóculo bacteriano, la mezcla se incubó en estufa bacteriológica por 5 minutos a 37ºC y posteriormente se sembró en medio de cultivo. El ácido peracético mostró eficiencia en la inhibición del crecimiento de *S. aureus* hasta una dilución de 10-2, siendo esta dilución al 0,001% de ácido peracético. En la dilución 10-3 hubo un crecimiento parcial del 44% con relación al grupo control, en las diluciones posteriores hubo un crecimiento bacteriano del 100%. El hipoclorito de sodio mostró eficacia hasta una dilución 10-4, correspondiendo esta dilución al 0,0001% de hipoclorito de sodio. En la dilución 10-5, el hipoclorito de sodio tuvo un efecto parcial de crecimiento bacteriano del 86 % en comparación con el grupo de control, en diluciones posteriores hubo un crecimiento bacteriano del 100 %. Se demostró que el hipoclorito de sodio es diez veces más efectivo que el ácido peracético para inhibir el crecimiento de *Staphylococcus aureus*.
Palabras clave: Ácido peracético; Hipoclorito de sódio; *Staphylococcus aureus.*

1. Introduction

Hospital infection (HI) is a very recurrent problem in public health. According to data from the World Health Organization (WHO), about 234 million patients are operated on each year worldwide, with 8 million acquiring nosocomial infections. In Brazil, it is estimated that HI reach about 14% of hospitalizations, aggravating the health status of patients, increasing hospitalization time and consequently generating higher expenses for the public health service.1 One of the microorganisms responsible for this is *Staphylococcus aureus,* which according to Svidzinski, et al. (2007) is usually found on our skin and mucous membranes, which occasionally can cause infections.3

The HI grows every day and something worrying is the form of resistance of *Staphylococcus aureus* to antibiotics. By means of a brief comparison, it can be said that bacteria are like the human body and antibiotics are like viruses. Viruses have the ability to kill a human being, but in the same way when a human being receives a vaccine, that is, he has a brief contact with the virus, he ends up sensitizing his immune system, becoming totally immune to it, and now it is no longer has more ability to kill you. In the same way as bacteria, antibiotics have the ability to eliminate them, but when they receive a brief contact with the antibiotic, they end up creating a means of adaptation to it, becoming immune to them as well.4

The exposure of antibiotics to *S. aureus* is often due to their indiscriminate use by people who self-medicate, ingesting antibiotics in an erroneous and irresponsible way, in addition to their excessive use in medicine, in the production of food for animals and in agriculture. These exposures to antibiotics sensitize bacteria without realizing it, creating even more organisms resistant to antibiotics, which in an earlier era were the solution to this problem.4 In this way, nosocomial infections become increasingly difficult to control. Prevention measures are taken by hospitals around the world, and the primary one would be adequate hand hygiene, since the hands are responsible for the spread of these bacteria within the hospital environment. The way to treat these infections is with the use of antibiotics, although *Staphylococcus aureus* has shown resistance to them since 1960, such as acquired resistance to oxacillin.5 Other ways of fighting *S. aureus* are with the use of chemical reagents such as hypochlorite sodium and peracetic acid, which according to Moura, et al., (2016) are excellent germicidal agents.

Substances called antimicrobials are substances that have the ability to inhibit bacterial growth or cause its death. The most famous antimicrobials are antibiotics, drugs that act directly to eliminate and inhibit the growth of bacteria, having the ability to inhibit protein synthesis, cell wall synthesis, DNA replication, among others. However, there are other substances considered antimicrobial, including sodium hypochlorite and peracetic acid, chemical reagents that have the ability to inhibit growth and eliminate bacteria.7

Peracetic acid has a high oxidizing power, promoting the oxidation of S-S and SH bonds of cellular components, which in turn directly affects the plasma membrane, deactivating physiological functions such as the osmotic barrier for example. Peracetic acid has rapid and high-level antimicrobial activity, being more effective than other antimicrobials, and as already mentioned, its decomposition is rapid, generating biodegradable and non-toxic products.8

Sodium hypochlorite (NaOCl) solution in contact with water can undergo dissociation, generating sodium hydroxide (NaOH) and hypochlorous acid (HOCl). When sodium hydroxide comes into contact with organic material, it reacts with amino acids to form salt and water. And hypochlorous acid (HOCl) similarly reacts with amino acids producing water and chloramines, and these interfere with bacterial metabolism.2,6,9 Large amounts of hydroxyls (OH-) are also released during this reaction. Increasing the amount of OH-- raises the pH of the solution, making the medium more basic. The elevation of this directly interferes in the “integrity of the cytoplasmic membrane with an irreversible enzymatic inhibition.10

In view of the health problems that *Staphylococcus aureus* has been causing society, being simpler infections, or more serious infections such as pneumonia, hospital infections are the most feared, as they worsen the clinical condition of several patients, increasing the risk of death. . Such infections can spread through the hospital environment, reaching a considerable number of patients. Most of the time, such outbreaks of infections are caused due to failures in the hygiene of the hospital professionals themselves.11

S. aureus, being a bacterium, is treated with the use of antibiotics, but it is famous for having a great resistance to antibiotics. Multidrug-resistant strains are called methicillin-resistant *Staphylococcus aureus* (MRSA), which have genes that guarantee resistance to antibiotics, which makes the fight even more difficult. Currently, hospital infections are usually controlled through excessive hand washing, isolation of infected patients and asepsis of patient rooms with sodium hypochlorite, peracetic acid, hydrogen peroxide, among other reagents with antimicrobial activity.12,13

Due to the increasing resistance acquired by *S. aureus* to antibiotics, it is necessary to use chemical reagents with germicidal activity for asepsis of instruments, benches and surfaces, as a way to prevent the spread of these microorganisms from one patient to another. The antimicrobial capacity of chemical reagents currently in use for hospital asepsis calls our attention, in addition to questioning which is the best reagent for such applicability.

A viable way of combating these microorganisms would be the use of these reagents with germicidal activity, which are easy to handle, have a low cost and do not present high risks to human health, such as peracetic acid, which also does not pose risks to human health. environment, because according to Svidzinski, et al., (2007) it is biodegradable.

Peracetic acid and sodium hypochlorite are great antimicrobials, capable of promoting the oxidation of cellular components and changes in cellular metabolism through the formation of chloramines.6 In this way, they will be well used for cleaning surfaces, becoming a great way to control nosocomial infections caused by *Staphylococcus aureus*.

With this, the objective of this work is to comparatively analyze the antimicrobial activity of peracetic acid and sodium hypochlorite against *S. aureus*, showing the best reagent in its inhibition, in addition to quantifying the minimum concentration necessary for growth inhibition.

2. Methodology

For the present work, two reagents were used, 0.2% peracetic acid and 2.5% sodium hypochlorite, both being stored according to the manufacturer's instructions. The sample of *Staphylococcus aureus* was donated by the microbiology sector of the Ruth Brazão clinical analysis laboratory, being isolated from a patient in the city of Belém-Pa. The bacterial strain was seeded in cled agar culture medium, kept in a bacteriological oven at 37ºC, after which *S. aureus* identification tests were carried out, using gram, catalase and coagulase staining tests. After identification, an inoculum of the bacteria was removed and mixed in a sterile saline solution.

For the test, the reagents had to be diluted, thus using 1% sodium hypochlorite and 0.1% peracetic acid, divided into experimental group I (peracetic acid) and II (sodium hypochlorite). For better quantification of bacterial colonies, pre-tests were performed to evaluate the best bacterial concentration to use in the work, the concentration of 10-2 was more favorable.
For the experimental group I, 10 microtubes were used receiving 900µL of the 0.1% reagent and serial dilutions from 10-1 to 10-9, for the experimental group II another 10 microtubes received 900µL of the 1% reagent and serial dilutions of 10-1 to 10-9. Another 100µL of bacterial inoculum mixed in saline solution was added to the microtubes of both groups, the mixture was homogenized and incubated in a bacteriological oven for 5 minutes at 37ºC.

For the positive control, 100µL of bacterial inoculum plus 900µL of saline solution were used, without the addition of chemical reagents, and for the negative control, 900µL of reagents and 100µL of saline solution were used. Both were incubated in an oven for 5 minutes at 37ºC. After this step, 10µL of the microtubes were dispensed into the cled agar culture medium, being spread with the aid of a microbiological loop. After growth, the plates were analyzed with the aid of a colony counter. The growth data of each plate in its respective dilution were tabulated, the test was performed in duplicate, with the two tests performed on different occasions, the control groups were submitted to the same conditions and techniques as the experimental group.

3. Results

The identification tests used had positive results for *Staphylococcus aureus*. We can check in Figures 1, 2 and 3, where respectively it can be verified by the positive catalase test that the bacterial genus was *Staphylococcus*, confirmed by the gram stain on a slide, where we observed that the bacterium is gram-positive, its morphology sphere (coconuts), and its arrangement in bunches of grapes (staphylo). Finally, the coagulase test to identify the bacterial species, in Figure 3, image A, we have a negative control to evidence a possible auto agglutination, and in image B we have the coagulase-positive result, confirming the bacterial species, this being *Staphylococcus aureus* (Table 1) .13,14,15

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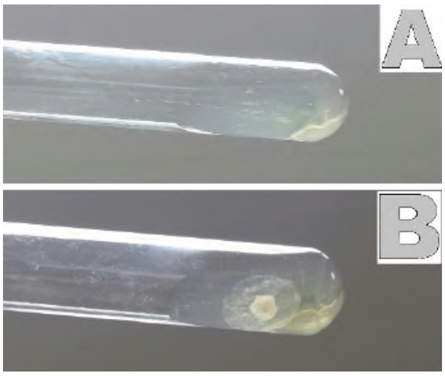
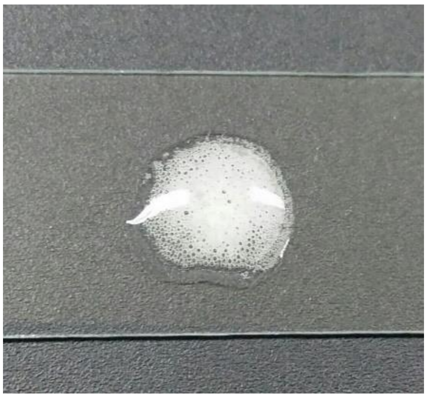
Figure 1. Negative (A) and positive (B) coagulase test.



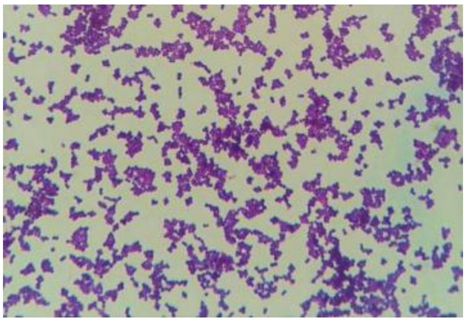
Source: Authors.

Figure 2. Positive catalase test.



Source: Authors.

Figure 3. Gram stain on slide.



Source: Authors.

For the experiment it was necessary to perform a dilution of the bacteria, since for a quantitative analysis it was necessary to count colonies. The dilution of the bacterial strain in 10-2 was chosen as ideal, because it was possible to obtain the largest number of colonies that could be counted. The type of sowing chosen was by dispersion, because in this way it is possible to obtain a spread and homogeneous growth of the colonies. The culture medium chosen was cled agar as it is an excellent culture medium for the growth of *Staphylococcus aureus*, having a good growth of deep yellow colonies.

Table 1. Antimicrobial action of peracetic acid and sodium hypochlorite on Staphylococcus aureusTrial 1.

Dilution	0,1%	10-1	10-2	10-3	10-4	10-5	≥10-6
Peracetic acid	N	N	N	*(44%)	P	P	P
Dilution	1%	10-1	10-2	10-3	10-4	10-5	≥10-6
Hypochlorite	N	N	N	N	N	*(86%)	P
Trial 2							
Dilution	0,1%	10-1	10-2	10-3	10-4	10-5	≥10-6
Peracetic acid	N	N	N	*(35%)	P	P	P
Dilution	1%	10-1	10-2	10-3	10-4	10-5	≥10-6
Hypochlorite	N	N	N	N	N	*(65%)	P
Average partial growth between trials		Peracetic acid 10-3 = 40%			Sodium hypochlorite 10-5 = 75%		

(N) no growth
(P) 100% growth compared to the control group
(*) Partial growth compared to the control group
Source: Authors.

In Table 1 we can see that peracetic acid showed total efficiency in inhibiting the growth of *S. aureus* up to a dilution of 10-2, being this dilution at 0.001% of peracetic acid. In the dilution of 10-3 there was a partial growth of 44% in relation to the control group, in the later dilutions from 10-4 onwards, the growth was 100% showing total ineffectiveness of the reagent at that concentration against *S. aureus*.

Sodium hypochlorite showed total efficacy up to a 10-4 dilution, this dilution being corresponding to 0.0001% sodium hypochlorite. At the 10-5 dilution sodium hypochlorite had a partial effect of 86% bacterial growth compared to the control group, at later dilutions there was 100% bacterial growth.

In the second test, as shown in Table 1, the same results of the first test were observed, showing the total confirmation of the experiment. Both 10-2 peracetic acid and 10-4 sodium hypochlorite exerted total inhibition of *S. aureus* growth. In the dilutions where the growth was partial, there was only a decrease in the value in relation to the control group, which was already expected, but the minimum concentration necessary was evidenced and confirmed with the second assay. In both assays the negative control groups confirmed the non-contamination of the experiment. The average partial bacterial growth of the reagents was 40% for 10-3 peracetic acid and 75% for 10-5 sodium hypochlorite.

In both assays, the positive control groups had 5 plates, where the average value of colonies was removed. In Figure 4 we can see a plate from the positive control group, showing the total growth of *S. aureus* free of any antimicrobial. In Figures 5 and 6, respectively, we have peracetic acid at 10-3 where we observed a partial inhibition of bacterial growth, about 44% compared to the control group, and sodium hypochlorite at 10-5, where we can also observe a partial inhibition of the *S. aureus*, about 86% compared to the control group.

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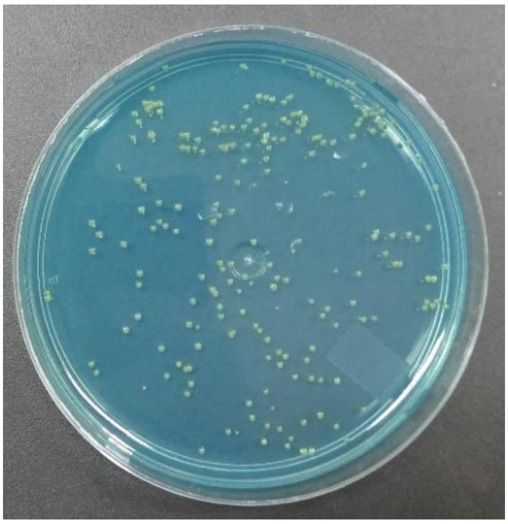
Figure 4. Positive control.

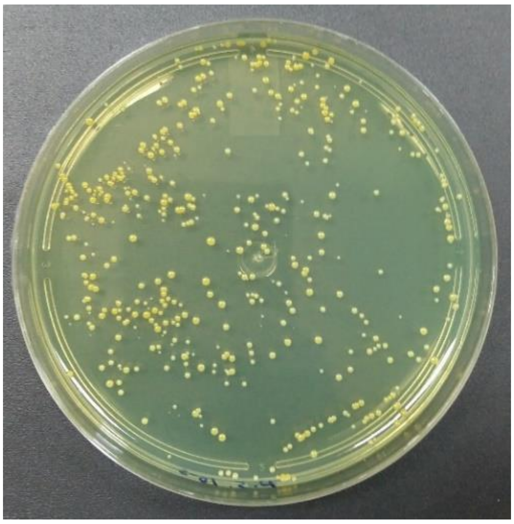
Source: Authors.

Figure 5. Peracetic acid $10^{-3.}$



Source: Authors.

Figure 6. Figure 5. Peracetic acid 10^{-5}



Source: Authors.

4. Discussion

Staphylococcus aureus was the bacterium chosen due to its importance in the context of hospital infection, being one of the most responsible for it, it is an aerobic bacterium, which multiplies rapidly, grows at room temperature, and usually lives in our skin and nasal cavities, being very easily propagated, where in contact with a weakened body it is fully capable of causing disease.2

An important feature in the control of nosocomial infections is to prevent the spread of the microorganism from an infected patient to the others. In addition to hand hygiene by health professionals who come into direct or indirect contact with patients proposed by the ministry of health, cleaning surfaces also becomes very important to prevent the spread of microorganisms. In the hospital environment, the use of chemical reagents such as antimicrobials for cleaning benches and surfaces is very common.16

The study by Svidzinski 2007, carried out in Maringá-PR using the bacterial strain of *S. aureus* as a sample, obtained results in which peracetic acid and sodium hypochlorite exerted the same efficacy. Based on the analysis of the results of the present work, it can be affirmed that the strain, from a patient hospitalized in Belém-PA, showed certain resistance to peracetic acid when compared with the results of the Svidzinski 2007. region, the bacterial strain in question in this work may have different characteristics from other strains, which explains the possible resistance to peracetic acid.2

5. Conclusion

Peracetic acid was shown to be very effective against *Staphylococcus aureus* up to a dilution of 0.001%, sodium hypochlorite showed great efficacy up to a dilution of 0.0001%, so sodium hypochlorite was ten times more effective than peracetic acid against *Staphylococcus aureus*, being the best reagent for its inhibition.

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