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Original Article

Evaluation of antibacterial and antifungal activity of antimicrobial soaps

Avaliação da atividade antibacteriana e antifúngica de sabonetes antimicrobianos

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Abstract

Soaps play an important role in our hygiene and health, as they not only have a bactericidal effect but also remove dirt from the human body. To evaluate the effectiveness of soaps with antimicrobial activity from different commercial brands sold in Brazil. Tests of the antimicrobial activity of different soaps were carried out through diffusion in agar against the microorganisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Candida albicans*. All commercial soaps tested transfer antimicrobial inhibition halo formation against *S. aureus* and *P. aeruginosa* bacteria. Only two commercial soaps inhibit the species *A. baumannii* and *C. albicans*. None of the seven products studied showed inhibition of *E. cloacae*, *P. mirabilis*, and *E. coli* bacteria. When comparing the information contained in the packaging of the products with the results obtained during a survey, divergences were observed. The soaps that provide greater efficiency against the tested microorganisms were presented in presentations 1 and 2, which become useful against the bacteria species *S. aureus, P. aeruginosa*, and *A. baumannii* and a fungus species *C. albicans*. Marks 3, 4, 5, 6, and 7 parallel the same sensitivity result opposite as bacteria of the species *S. aureus* and *P. aeruginosa*, with quantitative variation only of the inhibition halo. There was a divergence between the information contained in the packaging of the seven products under study and the results of the experimental tests.

Keywords: anti-bacterial agents, soaps, bacteria, *Candida* sp.

Resumo

Os sabonetes têm um papel importante para a nossa higiene e saúde, pois eles além de ter efeito bactericida, também removem as sujeiras presentes no corpo humano. Avaliar a eficácia de sabonetes com atividade antimicrobiana de diferentes marcas comerciais vendidas no Brasil. Foram realizados ensaios da atividade antimicrobiana dos diferentes sabonetes através do método difusão em ágar frente aos microrganismos *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Acinetobacter baumannii, Proteus mirabilis e Candida albicans.* Todos os sabonetes comerciais testados apresentaram formação de halo de inibição antimicrobiana frente às bactérias *S. aureus* e *P. aeruginosa.* Apenas dois sabonetes comerciais apresentaram inibição das espécies *A. baumannii* e *C. albicans*. Nenhum dos sete produtos estudados apresentou inibição das bactérias *E. cloacae, P. mirabilis e E. coli.* Na comparação das informações contidas nas embalagens dos produtos com os resultados obtidos durante a pesquisa realizada foram observadas divergências. Os sabonetes que apresentaram maior eficiência contra os microrganismos testados foram aqueles das apresentações 1 e 2, que se mostraram eficazes contra as espécies de bactérias *S. aureus, P. aeruginosa* e *A. baumannii* e contra a espécie de fungo *C. albicans*. As marcas 3, 4, 5, 6 e 7 apresentaram o mesmo resultado de sensibilidade frente as bactérias das espécies *S. aureus* e *P. aeruginosa*, com variação quantitativa apenas do halo de inibição. Houve divergência entre as informações contidas nas embalagens dos sete produtos em estudo e os resultados dos testes experimentais.

Palavras-chave: ação antibacteriana, sabonetes, bactérias, *Candida* sp.

1. Introduction

Soaps are considered the oldest hygienic products used by men (Arraes, 2018). They play an important role in hygiene and health, as in addition to having a cosmetic effect, they also remove dirt from the human body.

It is believed that the Greeks and Egyptians were the first to produce soaps. There is evidence of the production of a soap-like material that dates back to 2800 BC in ancient Babylon[1]. During the Middle Ages, the Catholic

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Church prohibited its use because it believed that exposing the skin, even while bathing, was demonic (Schutz et al., 2017). However, the need for sanitary measures and the evolution of knowledge about infections induced by bacteria and other microbial agents have valued the use of these products as a means of disease prevention.

In 1878, Irish chemist James Gamble began to manufacture a white, lightly perfumed soap that had a smooth, homogeneous consistency and could generate an enormous amount of foam. In the same period, the use of oils and seeds such as coconut, palm, and linseed began to replace olive oil as a raw material in the production of soaps. These are the first soap ever produced (Velozo, 2018).

The hygiene product called "soap" is a bar soap, usually perfumed, intended for body cleaning, and which may contain adjuvants, humectants, colorings, essences, and flavorings, among other additives. They are composed of alkaline salts of fatty acids resulting from a saponification reaction between an alkaline product with natural fatty acids and glycerides (Pereira, 2016).

Today, to meet an increasingly demanding, sophisticated consumer and globalized culture, changes and adaptations were necessary for the soap market. These changes created categories of soaps several uses and for a specific type or class of consumer (Souza et al., 2017).

According to the classification of personal care products and cosmetics by the National Health Surveillance Agency (ANVISA), soaps can be classified as Grade I or II. The Grade I category includes those products that have elementary properties, such as abrasive soaps or mechanical, facial, or body exfoliating soaps, and deodorant soaps. Grade II are products with specific indications, whose characteristics require proof of safety and/or efficacy, as well as information, care, mode, and restrictions of their use, such as antiseptic, children's, and intimate use soaps (Brasil, 2015).

The so-called antimicrobial soaps, disinfectants or antibacterial stand out in the cosmetic and personal hygiene market as the level of consumer demand for products with differentiated quality and better efficacy grows. These products generally have the cleaning, perfuming, and correction of body odor properties common to conventional soaps, and they have the purpose of preventing bacterial proliferation and infections related to pathogenic microorganisms (Costa et al., 2018). In this way, antiseptic soaps contribute to cleaning and perfuming the skin, intending to eliminate certain groups of microbes.

The human organism has a normal microbiota that comprises two distinct groups of microorganisms: the resident and the transitory. The resident microbiota is normally non-pathogenic, consisting of gram-positive bacteria and to a lesser extent gram-negative bacteria. The transient microbiota, originating from the environment and other areas such as the nasal mucosa and gastrointestinal tract, may contain different pathogenic microorganisms, such as *Pseudomonas, Enterobacter, Salmonella, Shigella*, and *Escherichia coli* (Gauer and Silva, 2017; Anjos et al., 2018).

Hand washing is extremely important for health care professionals due to the possibility of cross-contamination of opportunistic bacteria during the health care process (ANVISA, 2018). Therefore, studies show that soaps with antimicrobial actives remove more bacteria compared to other types of soaps (Baraldi, 2017; Costa et al., 2018; Leitzke et al., 2021).

Antibacterial soaps stand out in their packaging that eliminates 99.9% of microorganisms or bacteria. However, according to Rama et al. (2011), antibacterial soaps remove 65 to 85% of the population of microbes deposited on the surface of the skin from environmental sources and which can cause infections.

With the diversity of soaps that claim to have antimicrobial activity on the market, it is difficult for the consumer to choose a better brand. It is not known whether the products can eliminate bacteria during hand washing or even during a shower.

This research aimed to evaluate the antimicrobial activity of commercial soaps from different brands sold in Brazil.

2. Materials and Methods

2.1. Type of study

It is a laboratory study characterized by the analysis of the effectiveness of soaps with antimicrobial activity from different brands sold in Brazil.

2.2. Study location

The study was performed at the Microbiology Laboratory of the Cesmac University Center, located in Maceió, Alagoas.

2.3. Microorganisms used

One fungus isolate (*Candida albicans)* and six bacterial isolates (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Acinetobacter baumannii*, and *Proteus mirabilis*) provided by the Microbiology sector of the Clinical Analysis Laboratory from the Prof. Dr. Alberto Antunes University Hospital (HUPAA) of the Federal University of Alagoas (UFAL) were used.

The aforementioned hospital is located in the city of Maceió, Alagoas, Brazil. The microorganisms used in this study were identified using the Vitek® automated system. The choice of microorganisms was due to the ease of finding people with infections caused by pathogens in the routine of medical clinic services.

2.4. Soaps used

The acquisition of soap samples was carried out in commercial establishments located in different neighborhoods of the city of Maceió, Alagoas.

The selection of the sample of products for carrying out the tests took as reference information obtained in four commercial establishments in the city of Maceió about the best-selling brands and presentations during the research period.

Seven soap bars with antimicrobial activity from different brands and presentations were evaluated: Lifebuoy® (blue and red), Dettol®, Protex®, Floral Pro®, Protege®, and Biocrema®.

2.5. Laboratory tests

The yeast inoculum procedures used were adapted from the methods performed by Silva et al. (2016) and according to the CLSI document M44-A2 (CLSI, 2018a). The inoculum of bacterial species, in turn, was adapted and standardized according to the manual of the European Committee for Antimicrobial Susceptibility Assays (EUCAST, 2018) and the CLSI document M02-A12 (CLSI, 2018b).

2.6. Standardization of microbial suspensions

From the initial culture of colonies provided by the HUPAA/UFAL, an aliquot of the inoculum of each of the bacteria was transferred to a sterile saline solution adjusted in the turbidity to the 0.5 tube on the McFarland scale ($1.5x10^8$ UFC/mL) (Gauer and Silva, 2017). The same procedure was used for the composition of the fungal suspension sample, however, with turbidity adjustment up to tube 1.0 on the McFarland scale ($1.5x10^{16}$ UFC/mL) (EUCAST, 2018).

2.7. Preparation of soap concentrations

The quantities specified in Table 1 were used to prepare solutions containing each of the seven commercial soaps purchased.

Such products were mixed until obtaining a homogeneous solution.

2.8. Controls

For the negative control, a solution of sterile distilled water and ethanol in the proportion of 1:1 v/v was used. To avoid doubts about the interference of ethanol on the results measured for the microbial inhibition halos, ethanol was also added to the negative control.

200 µL of ampicillin solution (200 mg/mL) was used as a positive control for bacteria and 200 µL of ketoconazole solution (16 mg/mL) for the fungus (Ostrosky et al., 2008).

2.9. Agar diffusion technique

The agar diffusion technique is the indirect method for determining antimicrobial activity or the Kirby-Bauer method. The Kirby-Bauer test for antibiotic susceptibility has been a standard used in microbiology for years. This technique was first used in the 1950s by W. Kirby and A. Bauer and later standardized by the World Health Organization (WHO) in 1961.

In this study, the antimicrobial potential is presented in areas of growth inhibition, where the test microorganism is inoculated on a plate containing a solid culture medium. The product of interest is arranged at equidistant points on this same plate with the aid of a glass cylinder and, after incubation for 24 hours, it is observed whether there has been a growth inhibition halo around the substances (Reynolds, 2019).

In this work, therefore, the antimicrobial activity of commercial soaps at different concentrations was assessed using the Kirby-Bauer technique (Reynolds, 2019).

For its operation, six holes were made with a glass cylinder in the Müeller Hinton or Sabouroud agar culture medium previously inoculated with the microorganisms to be tested against the antimicrobial soaps.

200 µL of each concentration of commercial soap (200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL of each commercial soap) were transferred to each of the holes contained in the culture medium previously inoculated with the microorganisms of interest.

200 µL of ampicillin solution (200 mg/mL) was used as a positive control for bacteria and 200 µL of ketoconazole solution (16 mg/mL) for the fungus. All plates were incubated at 37 °C for 24 hours and all tests were performed in triplicate.

Table 1. Products and quantities used in the preparation of soap solutions.

Concentration	Soap (g)	Distilled water (mL)	Ethanol (mL)
200 mg/mL	4.00	18.00	2.00
100 mg/mL	2.00	19.00	1.00
50 mg/mL	1.00	19.50	0.50
25 mg/mL	0.50	19.75	0.25

2.10. Data analysis

The test results were grouped in an electronic database using the Microsoft Excel 2019 program and analyzed using descriptive statistics of absolute and relative frequency using the IBM SPSS *Statistics* 25.0 *software*.

2.11. Ethical aspects

In line with Resolutions 466/2012 and 510/2016 of the National Health Council - CNS/CONEP, as the data collection did not involve human beings, submission and/or approval by the Research Ethics Committee (CEP) was not necessary.

3. Results

The tests with the *S. aureus* bacteria showed a variation in the inhibition halo from 29.0 mm to 14.0 mm, with soaps 7 and 3 being more and less effective against this microorganism, respectively. The species *P. aeruginosa* presented a variation in the halo between 29.5 mm and 18.5 mm, with soaps 4 and 3 being more and less effective, respectively.

For the microorganisms *A. baumannii* and *C. albicans*, only soaps 1 and 2 showed microbial growth inhibition halos. *A. baumannii* showed a variation of antimicrobial halo for soap 1, in formulations 1a and 1b, and soap 2, in formulations 2a, 2b, and 2c. The halo variation for the species *A. baumannii* ranged from 22 mm to 17 mm, with products 2 and. The fungus *C. albicans* presented a variation of microbial growth inhibition halo for products 1 and 2 in formulations 1a, 1b, 2a, and 2b and its halo variation was from 30 mm to 20 mm, with soap 2 being more effective.

Table 2 presents the average of the results obtained in the antimicrobial activity assay by the agar diffusion method for the seven commercial soaps evaluated and their respective standard error values.

Table 2. Mean values of microbial growth inhibition halos of commercial soaps by the agar diffusion method.

	Inhibition halo diameters (*mm*)						
	S. aureus	***P. aeruginosa***	***E. coli***	***A. baumannii***	***E. cloacae***	***P. mirabilis***	***C. albicans***
S1							
S1 a	18 ± 0.0	29 ± 1.0	-	17 ± 0.0	-	-	26.5 ± 1.5
S1 b	19.5 ± 0.5	28 ± 2.0	-	18 to 0.0	-	-	24 ± 0.0
S1 c	17.5 ± 0.5	27 ± 0.5	-	-	-	-	-
S1 d	15 ± 0.0	25.5 ± 0.5	-	-	-	-	-
C^+	36 ± 0.0	15 ± 0.5	38 ± 1.0	19 to 0.0	22 ± 2.0	49.5 ± 0.5	49 ± 2.0
C^-	-	-	-	-	-	-	-
S2							
2 a	18.5 ± 0.5	28 ± 1.5	-	17 ± 0.0	-	-	31 ± 1.0
2 b	18.5 ± 0.5	26 ± 2.0	-	19 ± 0.0	-	-	20 ± 0.0
2 c	18.5 ± 0.5	23 ± 0.0	-	22 ± 0.0	-	-	-
2 d	17± 0.0	26.5 ±0.5	-	-	-	-	-
C^+	36.5 ±0.5	17± 1.0	38.5 ± 2.5	21 ± 0.0	29 ± 11.0	47 ± 2.0	49 ± 2.0
C^-	-	-	-	-	-	-	-
S3							
S3 a	15 ± 0.0	21 ± 4.0	-	-	-	-	-
S3 b	14.5 ± 0.5	21 ± 4.0	-	-	-	-	-
S3 c	14 ± 0.0	18.5 ± 0.5	-	-	-	-	-
S3d	14.5 ± 1.5	19.5 ± 3.5	-	-	-	-	-
C^+	35.5 ± 2.5	18 ± 2.0	38.5 ± 0.5	17 ± 1.0	17 ± 0.0	51.5 ± 0.5	49 ± 2.0
C^-	-	-	-	-	-	-	-
S4							
S4 a	16.5 ± 0.5	29.5 ± 1.5	-	-	-	-	-
S4 b	16.5 ± 0.5	27.5 ± 0.5	-	-	-	-	-
S4 c	16 ± 0.1	25.5 ± 0.0	-	-	-	-	-
S4 d	16 ± 0.0	25 ± 0.0	-	-	-	-	-
C^+	36.5 ± 0.5	22.5 ± 5.5	40.5 ± 0.5	21.5 ± 0.5	22 ± 0.0	54.5 ± 0.5	49 ± 2.0
C^-	-		-	-	-	-	-
S5							
S5 a	16.5 ± 0.5	20.5 ± 3.5	-	-	-	-	-
S5 b	16 ± 0.0	22.5 ± 5.5	-	-	-	-	-
S5 c	15.5 ± 0.5	19.5 ± 2.5	-	-	-	-	-
S5 d	16 ± 0.0	21 ± 3.0	-	-	-	-	-
C^+	38.5 ± 1.0	18 ± 0.0	43 ± 0.0	20.5 ± 0.5	21 ± 0.0	21 ± 0.0	49 ± 2.0
C^-	-	-	-	-	-	-	-
S6							
S6 a	15 ± 2.0	23.5 ± 2.5	-	-	-	-	-
S6 b	15.5 ± 1.5	21.5 ± 0.5	-	-	-	-	-
S6 c	17 ± 0.0	21.5 ± 0.5	-	-	-	-	-
S6 d	17 ± 0.0	24 ± 0.5	-	-	-	-	-
C^+	34.5 ± 2.5	17 ± 0.0	38 ± 2.0	15.5 ± 0.5	17 ± 0.0	50.5 ± 1.5	49 ± 2.0
C^-	-	-	-	-	-	-	-
S7							
S7 a	30 ± 0.0	21.5 ± 0.5	-	-	-	-	-
S7 b	29 ± 0.0	24 to 1.0	-	-	-	-	-
S7 c	28.5 ± 0.5	20.5 ± 0.5	-	-	-	-	-
S7 d	29 ± 0.0	21.5 ± 1.5	-	-	-	-	-
C^+	33.5 ± 1.5	18 ± 0.0	37.5 ± 0.5	20 ± 3.0	18.5 ± 0.5	36 ± 0.5	49± 2.0
C^-	-	-	-	-	-	-	-

S: commercial soap; (–): absence of inhibition halo; C^+: positive control; C^-: negative control. The numbers indicate the formulation (soap number); the letters indicate the concentration of the soap solution; a: 200 mg/mL; b: 100 mg/mL; c: 50 mg/mL; d: 25 mg/mL. Values are means of three determinations; ±: standard error of the mean of the inhibition halos.

Table 3 presents the profile of the effectiveness of antimicrobial soaps with the information on the label of commercialized products and the data obtained for sensitivity and antimicrobial resistance by the agar diffusion method.

All commercial soaps tested presented the formation of an antimicrobial inhibition halo against *S. aureus* and *P. aeruginosa* bacteria. Only two commercial soaps presented inhibition of *A. baumannii* and *C. albicans* species. None of the seven products studied presented inhibition of *E. cloacae*, *P. mirabilis* and *E. coli* bacteria.

When comparing the information contained in the packaging of the products with the results obtained during the research carried out, divergences were observed.

4. Discussion

The claim "*antiseptics*" is used for products with the property of preventing and/or inhibiting the growth of microorganisms on the skin and mucosa up to levels considered safe and within an adequate period. This class includes degerming soaps, antiseptics, products that contain alcohol in concentrations used for antisepsis, sanitizing products for domestic and hospital use, personal hygiene products, and mouthwash, among others (ABDI, 2015).

The choice of the ideal product has been of great interest to people, considering the diversity of products, the large market offer, and the variations in guidelines regarding indications and use (Toigo et al., 2020). Personal hygiene articles for this purpose must have a wide range of action, lower toxicity, lower cost. and less evidence of bacterial resistance (Alvarenga et al., 2007). Some are active against a large number of microorganisms, while others may only be active for one species. However, there is no ideal antimicrobial soap for all purposes (Costa et al., 2018).

The results showed the sensitivity and resistance profile of isolates of the microorganisms *C. albicans*, *Staphylococcus aureus, E. coli, P. aeruginosa, E. cloacae, A. baumannii*, and *P. mirabilis* to the cleaning agents tested.

Of the seven microorganisms, *S. aureus* and *P. aeruginosa* were the species most sensitive to the commercial soaps tested. All soaps tested showed the formation of an antimicrobial inhibition halo against *S. aureus* and *P. aeruginosa,* representatives of gram-positive and gram-negative bacteria, respectively. The variation in the size of the microbial activity inhibition halos observed may be due to the increase in viscosity resulting from the higher concentrations of soap. It is known that, when increasing the concentration of the product, there may be difficulties in its diffusion and an increase in the degree of interactions with the solid culture medium (ABDI, 2015).

S. aureus is normally found in healthy people, in the nasal passages and skin; however, if the natural barriers of these regions are compromised by trauma, surgery, or other purposes, this microorganism can generate pathogens through accommodation and dissemination in the tissue and the management of local injury. *S. aureus* triggers from simple infections like pimples, boils, and cellulitis to more serious conditions: pneumonia, meningitis, endocarditis, toxic shock syndrome, and septicemia. *P. aeruginosa*, which belongs to the soil microflora, can also come from the microbiota of the nasal cavities, mainly from farmers. It can be a pathogen with multiple antimicrobial resistance and that can cause infections of the varied clinical spectrum, such as dermatitis, urinary tract, and systemic infections, especially in immunocompromised patients, in addition to being an important cause of nosocomial infections generated during the care process (Valverde et al., 2018).

Table 3. Sensitivity profile of commercial antimicrobial soaps: label information versus laboratory test results.

Soap	Information	*S. aureus*	*P. aeruginosa*	*E. coli*	*A. baumannii*	*E. cloacae*	*P. mirabilis*	*C. albicans*
S1	Label	■		■				
	Assay	■	■		■			■
S2	Label	■		■				
	Assay	■	■		■			■
S3	Label	■	■	■				
	Assay	■	■					
S4	Label	■	■					
	Assay	■	■					
S5	Label	■	■	■				
	Assay	■	■					
S6	Label			■				
	Assay	■	■					
S7	Label		■	■				
	Assay	■						

S: commercial soap. Numbers indicate formulation (soap number).

This last species is considered one of the microorganisms with the greatest resistance to eradication, even when compared to other species from the gram-negative group such as *E. coli* (ABDI, 2015).

The results are in agreement with studies that also presented that *S. aureus* and *P. aeruginosa* strains were sensitive to a variety of commercially available antiseptic and antimicrobial products (Silva et al., 2016; ABDI, 2015; Araújo, 2013; Silva et al., 2018).

Although not common antimicrobial soaps sensitive to fungi, two commercial soaps tested in this study showed inhibition of *C. albicans*. The study developed, which aimed to evaluate the clinical effectiveness of soaps in controlling the biofilm present in dental prostheses, also found that some groups of soaps were effective in reducing *C. albicans* and *C. tropicalis*. In the case of *C. albicans*, in this study, the solutions of some groups of commercial soaps could bring the number of microorganisms to zero and to reduce the number of colonies of others, significantly. The author concluded that certain commercial groups of these products could be an alternative for disinfecting removable prostheses, taking into account their effectiveness in reducing biofilm (Tasso, 2019).

The same products that were effective against *C. albicans* are sensitive to bacterial strains of *A. baumannii*, a microorganism reported in the literature as being a multiresistant bacterium to the antibiotics available on the market (Gomes et al., 2016; Lima et al., 2019). The findings of these soaps lead to a probable conclusion of better efficacy.

None of the seven studied products showed inhibition of *E. cloacae*, *P. mirabilis*, and *E. coli*. These species are called Enterobacteriaceae and are part of a family of Gram-negative bacilli residing in the gastrointestinal tract, being characterized as multidrug-resistant organisms (Lukac et al., 2015). The antiseptic ineffectiveness of the tested products against this group is possibly linked to the multi-resistance capacity of this group of pathogens and their various adaptation mechanisms.

A limitation of this study concerns the difficulty in comparing the results with other studies that evaluated the ability of soaps to eliminate natural flora or artificially inoculated bacteria, either because of the differences related to the commercial varieties of the product formulations or because of the diversity of target bacteria adopted in the studies.

5. Conclusion

Through this study, it was possible to conclude that the soaps that showed greater efficiency against the tested microorganisms were those of presentations 1 and 2, which are effective against the bacterial species *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, and the fungus species *Candida albicans*.

Brands 3, 4, 5, 6, and 7 presented the same sensitivity result against *S. aureus* and *P. aeruginosa*, with quantitative variation only in the inhibition halo.

In none of the commercial soap formulations tested, there was the formation of an inhibition halo against the growth of *Escherichia coli*, *Proteus mirabilis*, and *Enterobacter cloacae*, concluding that these three bacteria are the most resistant to the commercial soaps studied.

There was a divergence between the information contained on the labels of the seven products studied and the results of the experimental tests.

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