

ORIGINAL ARTICLE

Studies On Bactericidal Activity Of Different Soaps Against –
Bacterial Strains

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ABSTRACT

The great majority of the studies on the effect of antibacterial soaps on the flora of human skin have been carried out on hands because of the interest in determine in surgical scrub procedures and because hands carry large numbers of microorganisms and permit the demonstration of sizable reduction in numbers. Soaps are the combination of fats and oils (of animal or vegetable origin) and Salt. Dermatological bars or cakes and disinfectants are chemical of different from soaps and contain modified detergents to enhance their use for antibacterial activity. Soaps and disinfectants is necessary in order to establish that which produce clean hands sufficiently in the easiest and most acceptable manner for specific food operation facilities The aim of this work to compare the efficacy of locally available market soaps against some bacteria such as *Staphylococcus* spp., *Pseudomonas* spp. and *Serratia* spp. as well as to provide data to clinician to decide for the selection of better and protective soap against pathogenic microorganism.

Key word: Disinfectants, *Staphylococcus* spp., *Pseudomonas* spp. *Serratia* spp.

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INTRODUCTION

Soaps are the combination of fats and oils (of animal or vegetable origin) and Salt [1]. Dermatological bars or cakes and disinfectants are chemical of different from soaps and contain modified detergents to enhance their use for antibacterial activity. The great majority of the studies on the effect of antibacterial soaps on the flora of human skin have been carried out on hands because of the interest in determine in surgical scrub procedures and because hands carry large numbers of microorganisms and permit the demonstration of sizable reduction in numbers[2]. Microorganisms carried on the skin of the human body divided into two distinct populations resident and transient[3]. Transient microorganisms are found within the epidermal layer of skin and these belong to the disease producing microorganism. Pathogens such as *E.coli*, *Salmonella* spp., *Shigella* spp., *Clostridium perfringens* and *Hepatitis A virus* may present on the skin. An antibacterial soap can remove 65% to 85% of bacteria from human skin [4]. Antibacterial soap and disinfectants are used as an adjunct to acne treatment, since they contain bacteriostatic agents. Detergency of soaps and disinfectants is another important factor in removing transient microorganisms from hands. Soaps and disinfectants is necessary in order to establish that which produce clean hands sufficiently in the easiest and most acceptable manner for specific food operation facilities[5].

Soaps containing agents active against gram positive organisms in reducing the incidence of pyogenic skin infection attributed primarily to *Staphylococcus aureus* associated with as increased susceptibility to skin infections with gram-negative species. The study of the influence of adlib use of an antibacterial soap on the total aerobic bacterial flora on six skin sites [6]. A chemical widely used to make soap “antiseptic” survives sewage treatment and in being spread onto farmland and released into water. When any antimicrobial is widely used or released, organisms have the potential to evolve resistance to its

effects. Everyone agree that warming our hands is good, but there is little difference between using soap and using antimicrobial soap. Disinfectants are substances that are applied to inanimate objects to destroy harmful microorganisms. The choice of disinfectant agent is based on the intended use of the patient care item. Preservatives are incorporated into medication or fluids to prevent microbial growth. A variety of chemical agents are used as disinfectants; the choice of an agent depends on its intended use. The agents currently approved for use as high-level disinfectants, (e.g) chlorine, peracetic acid, and ortho-phthalaldehyde.

Alcohol is widely used for the environmental disinfection of small areas (i.e.) "spot" disinfection. Alcohol is not effective as a surface disinfectant against *adenovirus* and its use to disinfect tonometer tips has been associated with epidemic kerato conjunctivitis. Formaldehyde inactivates microorganisms by alkyl the amino and sulfhydryl groups of proteins and the ring nitrogen atoms of purine bases. Formaldehyde (8.0 g/dl) used for disinfection of surfaces and infusion pumps while an outbreak of *Pseudomonas spp* due to deficient formaldehyde mixing used to disinfect dialyzer was reported. Antiseptic hand rubs are waterless agents with disinfectant properties that decrease the number of microorganisms present. Most alcohol based hand antiseptics contain either isopropanol, ethanol, n-propanol or a combination of these two products [7]. Hand hygiene is an important infection control intervention to prevent the transmission of microorganisms. Visible organic material must be removed for waterless agents to exert their antimicrobial activity [8]. Methicillin – resistant *Staphylococcus aureus* (MRSA) continues to be a global problem in infection control. The proportion of methicillin resistance among clinical isolates is of *S. aureus* is still increasing study was to determine the efficacy of the antiseptic soap Stellisept scrub in combination with mupirocin for eradication of MRSA [9]. In this present investigation study to compare the efficacy of the commercially available soaps and pathogenic microbes.

MATERIALS AND METHOD

To perform this experimental study different soaps of common use from shops were purchased and their dilution were made for testing the bactericidal activity of different organisms.

Collection of Test Sample

The samples were collected from skin infection patients of the hospital.

Sampling Procedure

Samples were collected from the skin infected patients by using sterile cotton swab and kept inside a sterile screw cap tubes containing 1% peptone broth. The swabs collected are transported to the laboratory.

Isolation of Organisms

Then the samples were streaked on Cetrimide agar, Mannitol salt agar, DNase agar, and Barid Parker agar plate. The results were recorded all the isolates were stored on Nutrient agar slants, Broth cultures were prepared for under going further methods.

List of Soaps

1. Lifebuoy, 2. Dettol, 3. Medimix, 4. Savlon, 5. Johnson's Baby

Preparation of Sterile Disc

Whatman's No.3 filter paper were made in 5mm disc form and wrapped in aluminium foils and sterilize in an oven for 30 minutes. Each sterile disc was incorporated individually with 10 to 20 µl of soaps. These discs were allowed for air drying.

Assay of Antimicrobial Activity

Overnight cultures were kept ready for antibiotic assay. Assay of the antimicrobial activity of soaps was done by the following method.

- Disc Diffusion Method
- Agar Diffusion Method
- Minimum Inhibitory Concentration Technique.

Disc Diffusion Method

Using proper sterile technique, the Muller Hinton Agar plates were prepared. Dip a sterile swab into the dilute bacterial solution and then swab it gently across the plate. Sterile filter paper disc loaded with soaps were placed on the center of each of the marked sections of Muller Hinton agar plates. Use a forceps to gently press each disc against the agar surface to ensure good contact. For the control plates apply sterile discs dipped in sterile water to each of the marked sections. Then the plates were incubated at 37°C ± 0.5°C for overnight. The zone of inhibition was measure and recorded.

Agar Diffusion Method

The Muller Hinton Agar was prepared sterilized and 20 ml of media was aseptically poured on petriplates. After solidification other parts of Muller Hinton Agar at pourable temperature was mixed

with test microbial strains and poured over solidified agar. After solidification five wells were made in each plate to each bacterial strains. The wells are incorporated with 20 µl of different soaps. Then the plates were incubated at 37°C±0.5°C for 12 to 14 hours without any disturbance. The zone of inhibition was measured and recorded.

Dilution of Soaps

Dilute the soaps into 5 µl and 10 µl concentration. After that the Muller Hinton agar plates were prepared, sterilized and 20 ml of media was poured on petriplates. After solidification other parts of Muller Hinton agar at pourable temperature was mixed with test microbial strains and poured over solidified agar. After solidification wells were made each well incorporated 20 to 30 µl of serially diluted soaps and further incubated at 37°C ± 0.5°C for 12 to 24 hours. The zone of inhibition was determined by measuring the diameter in millimeters of zone to which the soap inhibited the growth of the organism.

Minimum Inhibitory ConcentrationThe minimum inhibitory concentration [MIC] was determined against the pathogens by serial dilution technique.

Preparation of seed culture

Each test organisms were inoculated into the Muller Hinton Broth and incubate 37°C ± 0.5°C for 4 to 6 hours. Divide the soap plate into different sector and inoculate spot inoculums on the plate with each organism and further incubate the plate 37°C ± 0.5° for 18-24 hours. After incubation the plates were observed and the results were recorded. In the Muller Hinton Broth, test organism and different soaps were inoculated at various concentrations such as 0.5 ml, 0.7 ml, 0.8 ml and 1 ml and it was incubated at 37°C for 24 hours and their OD values were recorded.

RESULTS

The evaluation of Medimix against *Pseudomonas* shows maximum activity at 1000 µl of 0.562 against the minimum activity at 500 µl of 0.334. Fig.2 shows for the evaluation of Dettol against *Pseudomonas* which has maximum activity at 1000 µl of 0.484 against the minimum activity at 500 µl of 0.248. Data revealed against *Pseudomonas* in the evaluation of Lifebuoy with maximum at 1000 µl of 0.372 against the minimum at 500 µl of 0.228 which shown in (Fig 3). The inhibitory activity of Savlon against *Pseudomonas* which shows maximum activity at 1000 µl of 0.367 against the minimum activity at 500 µl of 0.188. (Fig.4). Fig 5 described the effectiveness of Johnson's Baby against the selected *Pseudomonas* according to that 1000 µl concentration shows the maximum activity of 0.284 against the minimum activity at 500 µl of 0.076.

The inhibitory activity of Medimix against the test organism of *Staphylococcus* shows the maximum activity at 1000 µl of 0.265 against the minimum activity at 500 µl of 0.222 in (Fig 6). In Fig 7 the evaluation of Dettol against *Staphylococcus* shows the maximum activity at 1000 µl of 0.087 against the minimum activity at 500 µl of 0.014. The soap Lifebuoy shows maximum activity against *Staphylococcus* of 0.097 at 1000 µl and minimum activity at 500 µl of 0.043 in (Fig 8). The value remarked against *Staphylococcus* in the evaluation of Savlon maximum activity at 1000 µl of 0.306 and shows minimum activity at 500 µl of 0.163 in (Fig 9). The result of Fig.10 in the evaluation of Johnson's Baby *Staphylococcus* shows the maximum activity at 1000 µl of 0.376 and shows the minimum activity at 500 µl of 0.106.

Data revealed on *Serratia* in the evaluation of Medimix shows maximum activity at 1000 µl of 0.27 minimum activity at 500 µl of 0.232 in Fig 11. Dettol shows activity against *Serratia* with maximum activity at 700 µl of 0.5 and minimum activity at 500 µl of 0.026 in Fig 12. In Fig 13 the evaluation of Lifebuoy against *Serratia* shows maximum activity at 1000 µl of 0.332 and minimum activity at 500 µl of 0.072. The inhibitory activity of Savlon against *Serratia* which shows maximum activity at 1000 µl of 0.288 and minimum activity at 500 µl of 0.146 in Fig 14. The value determined in Fig.15 in the evaluation of Johnson's Baby against *Serratia* which shows maximum activity at 1000 µl of 0.228 and minimum activity at 500 µl of 0.074.

In this method Muller Hinton agar plates were prepare and poured and the well was plugged. In agar diffusion method Medimix shows maximum zone of inhibition against *Pesudomonas* with a diameter of 1.2 mm and minimum zone in Lifebuoy of 0.3 mm in Fig 16. Soap Medimix shows maximum zone of inhibiton against *Pesudomonas* with a diameter of 0.4 mm and minimum zone in Johnson's Baby and Savlon at a diameter of 0.2 mm in the Disc diffusion method Fig 17.

In the MIC method at the concentration level of 10 µl of Medimix against *Pesudomonas* shows maximum zone at 0.9 mm and Savlon shows minimum zone at 0.2 mm in Fig 18 and in the 5 µl of different soaps against *Pesudomonas* Dettol shows higher zone at 0.6 mm and lower zone in Johnson's Baby Fig 19. In fig 20 against the *Staphylococcus* spp in well method of the 5 different soaps Medimix shows maximum zone at 1 mm and minimum in Savlon at 0.5 mm. Of the 5 different soaps according to the disc method against

Staphylococcus, Medimix showed maximum zone at 0.7 mm and minimum zone of Savlon and Johnson's Baby at 0.4 mm (Fig 21) Data revealed in the MIC method at the concentration level of 10 µl, soaps against *Staphylococcus*, Medimix shows maximum activity of zone at 0.8 mm minimum of Savlon and Johnson's Baby at 0.1 mm (Fig 22). The result of fig.23 showed that in MIC method at the concentration of 5 µl of different soaps against *Staphylococcus*, Medimix shows maximum zone at 1.1 mm and minimum in Johnson's Baby at 0.1 mm. According to the agar diffusion method among the different soaps against *Serratia*. Medimix shows the maximum some at 0.8 mm and Lifebuoy shows the minimum zone at 0.5 mm (fig.24). Fig. 25 determined, according to the disc method against *Serratia*, Medimix shows maximum zone formation at 0.6 mm and minimum in Lifebuoy at 0.3 mm. Medimix shows maximum zone in the concentration level of 10 µl in MIC method against *Serratia* at diameter of about 1.4 mm and minimum zone at 0.2 mm in Savlon and Johnson's Baby in Fig.26 and at the concentration level of 5 µl against *Serratia* shows maximum zone in Medimix at 1.3 mm and minimum zone in Johnson's Baby at 0.4 mm in fig.27. According to the Antibiotic sensitivity test against *Pseudomonas* Ciprofloxacin shows more effective at a diameter of 2.8 mm and Trimethoprim and Penicillin shows less effective in Fig.28. In fig.29 shows that Chloramphenicol shows more effective against *Staphylococcus* at a diameter of 2.8 mm and Colistin shows less effective at 1mm. Data revealed in fig.30 shows the antibiotic sensitivity test against *Serratia* shows that Cephalexin and Ciprofloxacin shows more effective at 2.6 mm and Ampicillin, Trimethoprim, Colistin, Penicillin G (0.8 mm) shows less effective against *Serratia* spp.



FIG. 20 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY WELL METHOD IN *Staphylococcus*

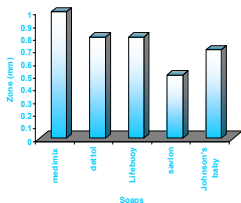


FIG. 21 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY DISC METHOD IN *Staphylococcus*

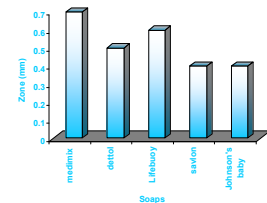


FIG. 22 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY MIC METHOD (10 µl) IN *Staphylococcus*

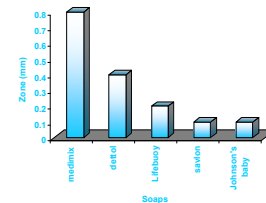


FIG. 23 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY MIC METHOD (5 µl) IN *Staphylococcus*

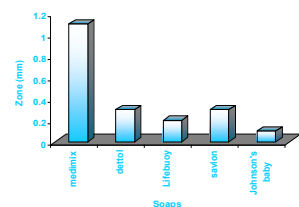


FIG. 26 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY MIC METHOD (10 µl) IN *Serratia*

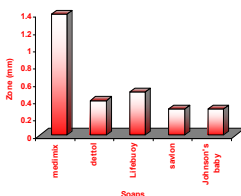


FIG. 27 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY MIC METHOD (5 µl) IN *Serratia*

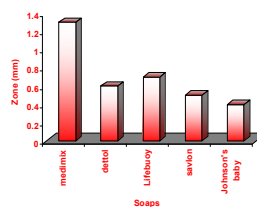


FIG. 25 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY DISC METHOD IN *Serratia*

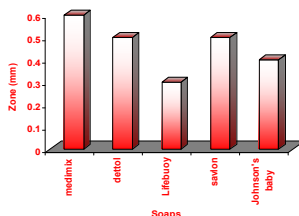


FIG. 24 EVALUATION OF ANTI-BACTERIAL ACTIVITY OF SOAPS BY WELL METHOD IN *Serratia*

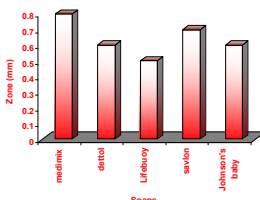


FIG. 28 SENSITIVITY TEST BY ANTIBIOTIC AGAINST *Pseudomonas*

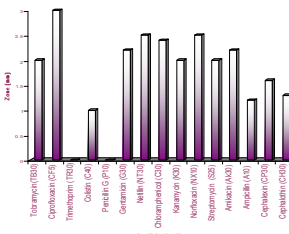


FIG. 29 SENSITIVITY TEST BY ANTIBIOTIC AGAINST *Staphylococcus*

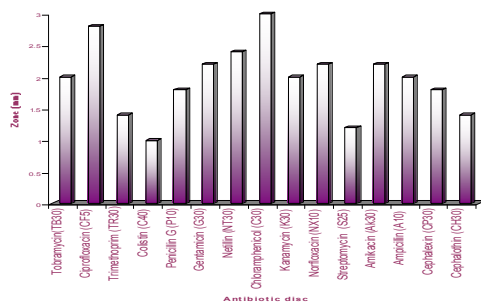
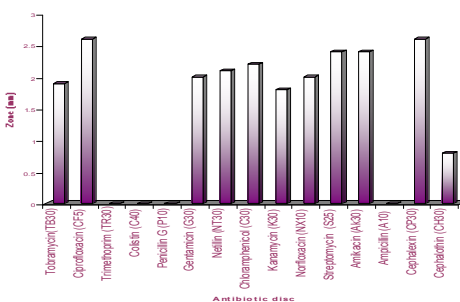


FIG. 30 SENSITIVITY TEST BY ANTIBIOTIC AGAINST *Serratia*



DISCUSSION

In present study isolate, *Staphylococcus spp.*, *Pseudomonas spp.*, and *Serratia spp.* was used to test the efficacy of antimicrobial soaps (Medimix, Dettol, Lifebuoy, Savlon, Johnson's Baby) were compared. According to Bamber [10], *S.aureus* was used to test the efficacy of antimicrobial soaps such as (Safeguard, Dettol, Lifebuoy, Johnson's Baby) deodorant soap (Lux), plain soap (Sufi and Sunlite) and disinfectant (Phenol) were compared. When efficacy of antibacterial soaps was compared Medimix was found to be more effective against *Pseudomonas* (0.562) and Johnson's Baby was found to be more effective against *Staphylococcus* (0.376) than all other antibacterial soaps. But Dettol was found to be more effective against *Serratia* (0.5) than all other soaps. Safeguard was found to be more effective against *S.aureus* than all other soaps. This study was supported by study of. In Minimum Inhibitory Concentration (MIC) method various concentration such as 0.5 ml, 0.7 ml, 0.8 ml and 1 ml were used. According to this Medimix shows higher activity at the concentration of 1000 µl. When efficacy of five different soaps were compared, MIC of Medimix against *Staphylococcus* was 0.8 mm in 10 µl, Dettol against *Staphylococcus* was 0.4 mm in 10 µl, Lifebuoy against *Staphylococcus* was 0.2 mm in 10 µl. Savlon and Johnson's Baby against *Staphylococcus* was 0.1 mm in 10 µl. In MIC of 5 µl Medimix against *Staphylococcus aureus* was 1.1mm, Dettol against *Staphylococcus* was 0.3 mm, Lifebuoy against *Staphylococcus* was 0.2 mm, Savlon against *Staphylococcus* was 0.3 mm and Johnson's Baby against *Staphylococcus* was 0.1 mm. This shows that Medimix exhibited more active against *Staphylococcus* than Dettol, Lifebuoy, Savlon and Johnson's Baby. When efficacy of deodorant soap (Lux) and plain soaps (Sunlite and Sufi soap) was compared, MIC of Sufi soap against *S.aureus* was 10240 µg/ml, and MIC of Sunlite was 20480 µg/ml, whereas MIC of Lux was 24576 µg/ml. This showed that Sufi soap and Sunlite

exhibited more activity against *S.aureus* than Lux [11]. When antibacterial soaps used properly, these washes may effect a reduction in *P.acnes* and prevent secondary infections in acne skin [12]. Medimix shows more effective against *Pseudomonas spp.* and *Staphylococcus spp.* Dettol soap was found to be more effective against *Serratia spp.* Dettol soap was also effective against *S.aureus* but its efficacy against *S.aureus* was less than that of Safeguard. Antibacterial activity of Johnson's Baby soap was comparable to that of Medimix, Dettol, Savlon and Lifebuoy, but it was less effective against all the 3 spp. such as *Pseudomonas*, *Staphylococcus* and *Serratia spp.* According to Malik Sattar Bakhsh *et al* (2006), antibacterial activity of Johnson's Baby soap was comparable to that of Safeguard and Dettol soap, but it was less effective than Safeguard against *S.aureus* but more than that of Dettol. The ingredients include in Johnson's Baby soap was sodium palm Kernelate, Sodium Palmate, Water, Mineral oil, Fragrance, Glycerin, Titanium Dioxide, Dimethicone, C12 - C15 Alkyl Benzoate, Stearoytrimethylsilane, Stearyl alcohol, Disodium EDTA, Hydrolyzed Milk Protein, Tocopheryl Acetate. Johnson's Baby soap care baby's delicate skin needs in growing years. Dettol soap gives 10 times better protection against a wide range of germs. The key ingredients such as Sodium soap, Fragrance, TCC, Colour, Preservative. Savlon soap also used as a liquid hand wash which gives gentle protection and active ingredient such as Triclosan may be present. Lifebuoy shows balanced germ protection and which is made from 100% vegetable oils. Medimix soap shows more effective for dry skin, it contains a unique combination of the purest and finest grade vegetable glycerine and the time tested Ayurvedic formulation of Lakshadi Oil. Of the 15 antibiotic disc such as Tobramycin, Ciprofloxacin, Trimethoprim, Colistin, Penicillin G, Gentamicin, Netillin, Chloramphenicol, Kanamycin, Norfloxacin, Streptomycin, Amikacin, Ampicillin, Cephalexin, Cephalothin among this Ciprofloxacin shows more effective against *Pseudomonas*. Trimethoprim and Penicillin G shows less effective. In *Staphylococcus spp.* Chloramphenicol shows more effective and Colistin shows less effective and Cephalexin and Ciprofloxacin shows more effective against *Serratia spp* and Colistin, Penicillin G, Trimethoprim and Ampicillin shows less effective in skin infection pathogens. Among the 10 antibiotics such as Oflaxacin, Enaxacin, Gentamicin, Enoxacin, Tobramycin, Norfloxacin, Pipemidic acid cefclor co - trimaxazol, Nalidexic acid, Amoxicillin, Cefazolin. Oflaxacin was more effective against both Gram negative bacilli and Gram positive cocci. Enaxacin and Gentamicin were equally effective against Gram-positive urinary tract isolates, while Enoxacin was more effective than Gentamicin against Gram-negative bacilli. Tobramycin was the second most effective against Gram-negative bacilli Co-tromoxazole was 47.88% and 42.86% effective against both Gram-positive and Gram-negative urinary tract pathogens [13]. Chloramphenicol has more effective against *Staphylococcus* comparable to that of Colistin. Chloroxylenol has effectiveness comparable to that of Triclosan [14]. Norfloxacin was more effective than Kanamycin in skin infection sample. According to Norrby (1987), Norfloxacin was extremely effective against urinary tract isolates.

CONCLUSION

Hand rubbing with an alcohol based solution is more effective than hand washing with an antiseptic soap in reducing bacterial contamination of health care workers hands during routine patient care found that hand rubbing was equivalent to antiseptic hand washing in reducing hand contamination. A prospective bactericidal activity of various market soaps was performed against bacterial strains i.e. *Staphylococcus spp*, *Pseudomonas spp* and *Serratia spp* to ascertain the efficacy of different soaps in daily use. Minimum inhibitory concentration (MIC) were determined by micro dilution technique. Among the five soaps such as Lifebuoy, Dettol, Medimix, Savlon and Johnson's Baby, the highest efficacy was performed by Medimix. Second most effective soap was Dettol. Third most effective soap was Lifebuoy where as Savlon and Johnson's Baby exhibited similar activities, among this Johnson's Baby is the less effective while comparing all other soaps. The bactericidal activity of these soaps were in increasing order in Medimix, Dettol, Lifebuoy, Savlon and Johnson's Baby respectively. Prevention concluded that alcohol based hand rubs are more effective than washing hands with antimicrobial or non-antimicrobial soap, can be made more accessible require less time to use and are less prone to cause irritant contact dermatitis.

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