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ORIGINAL PAPER

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Antimicrobial effects of Burow's solution on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract Burow's solution has been shown to be effective against chronic suppurative otitis media and otitis externa. We demonstrated that Burow's solution had antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, inducing ultrastructural changes in these bacteria in vitro. *S. aureus* strain 209P and *P. aeruginosa* strain IID1130 were treated with 13% Burow's solution. Viable cell counts were determined to measure bactericidal effects. Ultrastructural changes in cells of both strains were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Viable cell counting revealed that *S. aureus* cells treated with Burow's solution were killed within 30 min. The viable cell count of *P. aeruginosa* was reduced by 1×10^7 colony-forming units/ml (CFU/ml) after a 60-min treatment. SEM examination of *S. aureus* revealed blebbing on the surface of bacterial cells, whereas TEM revealed undulating deformation of the bacterial cell wall, diluted cytoplasm, and cell membrane detachment. SEM observations of *P. aeruginosa* revealed a more apparent undulating deformation of the bacterial cell surface. TEM observations also revealed deformations in the bacterial cell wall and diluted cytoplasm in both bacteria. These findings show that Burow's solution is active against *S. aureus* and *P. aeruginosa*, resulting in damage to the cell wall.

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Key words Antimicrobial effect · Burow's solution · Ultrastructural examination· *Staphylococcus aureus* · *Pseudomonas aeruginosa*

Introduction

Chronic otitis media is a commonly encountered chronic inflammation of the middle ear and mastoid process with a perforated tympanic membrane and discharge.[1] Otitis externa, an infection of the external auditory canal, is associated with exposure to warm humid climates, swimming, and aggressive cleaning of the ear canal.[2] These diseases, which are common in primary care, are difficult to cure because of the spread of antibiotic-resistant bacteria.[3]

Burow's solution, named after Karl August von Burow, has been used as a local otological preparation in the treatment of chronic otitis media and external otitis media since the 19th century.[4] This colorless liquid with a slight acetic acid odor is composed of approximately 13% aluminum acetate with a pH of 3.1. It has long been believed that the solution has antimicrobial and astringent effects. Recently, Thorp et al.[4] and Terayama et al.[5] reported clinical efficacy for chronic otitis media and determined the optimal dilution for clinical usage. Clinically, it has been reported that Burow's solution is effective for external otitis and chronic otitis media.[6,7] Because there are few reports[6] examining the antimicrobial effects of Burow's solution, we have no relevant data regarding its antimicrobial activity.

In the present study, we attempted to clarify the antimicrobial activity of Burow's solution against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which cause otitis externa and otitis media, respectively.

Materials and methods

Bacterial strains and media

Staphylococcus aureus strain 209P (IID671) and *P. aeruginosa* strain IID1130 were used in various experiments.

Bacterial cells cultured in trypto-soya broth (TSB; Nissui Pharmaceutical, Tokyo, Japan) were inoculated into 20 ml fresh medium and incubated at 37°C with continuous agitation for 18 h.

Antimicrobial effects of Burow's solution

Bacterial cells of *S. aureus* and *P. aeruginosa* were incubated at 37°C overnight. Cells were then harvested, washed twice with Dulbecco's phosphate-buffered saline (PBS), suspended in 0.1 ml PBS, and inoculated into 0.9 ml 13% Burow's solution, which was prepared at Kawasaki Medical Hospital. Samples were taken periodically and spread on nutrient agar without antimicrobials. Following overnight incubation at 37°C, viable cells were counted and recorded in terms of colony-forming units/ml (CFU/ml).

Electron microscopy

After a 15-min incubation in Burow's solution, bacterial cells were harvested. After washing twice in PBS, the collected cells were processed for electron microscopy as previously described.[8–11] Sample preparation for scanning electron microscopy (SEM) included several washes of cells with PBS; then, a drop of bacterial suspension was mounted on a glass coverslip and fixed twice with 2.5% glutaraldehyde and 1.0% OsO_4. After dehydration through an ethanol series, the samples were dried by the *tert*-butyl alcohol freeze-drying method. Dried samples were mounted, sputter-coated with platinum, and examined using a JEOL JSM-6340F scanning electron microscope (SEMTech Solutions, North Billerica, MA, USA) at 25 kV. Transmission electron microscopy (TEM) was performed for ultrastructural characterization of cells. Bacterial cells were incubated at 37°C for 18 h in TSB (Nissui Pharmaceutical), harvested, washed twice with PBS, pelleted, and fixed in 2.5% glutaraldehyde followed by 1.0% OsO_4. The specimens were dehydrated by passing through an ethanol series and embedded in Spurr's Epon resin. Ultrathin sections were cut on an ultramicrotome with a diamond knife, stained with uranyl acetate and lead citrate, and examined under a JEOL JEM-2000EXII transmission electron microscope (SEMTech Solutions) at 80 kV.

Results

Antimicrobial activity of Burow's solution

The bactericidal effects of Burow's solution against *S. aureus* 209P and *P. aeruginosa* IID1130 were measured over time by viable cell counting (Fig. 1). The viable cell count of *S. aureus* was reduced to $1/10^3$ CFU/ml after 15 min of treatment with Burow's solution, and all bacterial cells were killed within 30 min. The viable cell count for *P. aeruginosa* remained unchanged after 15 min of treatment but was reduced to $1/10^3$ CFU/ml after 40 min and to $1/10^7$ CFU/ml after 60 min. These findings demonstrate the bactericidal effects of Burow's solution against both *S. aureus* and *P.*

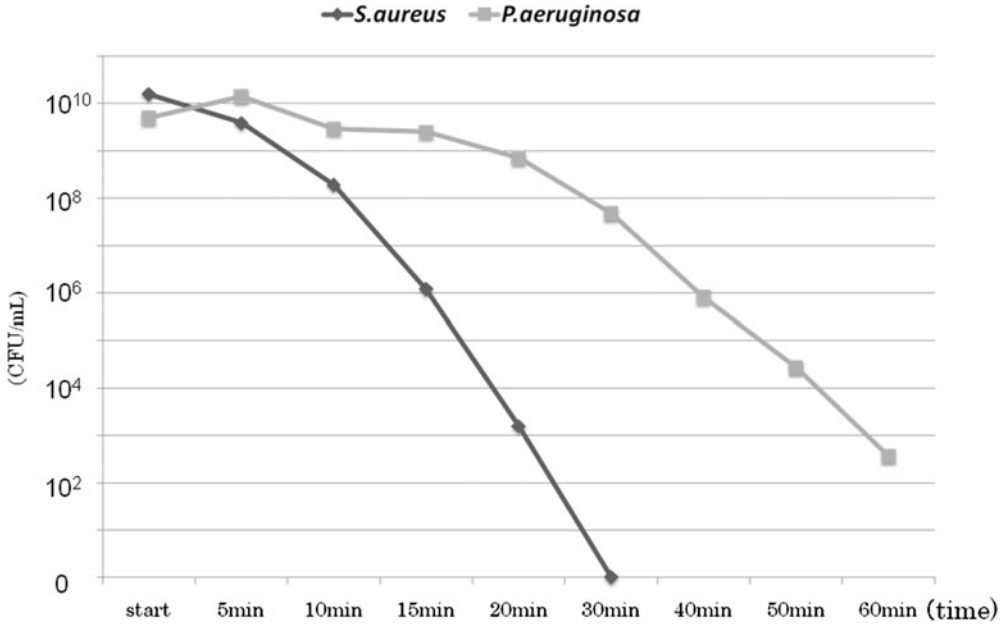

Fig. 1. Antimicrobial activity of Burow's solution against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). Bacterial cells were incubated at 37°C overnight, harvested, suspended in 0.1 ml phosphate-buffered saline (PBS), and inoculated into 0.9 ml 13% Burow's solution. Periodic samples were spread on nutrient agar without antimicrobials. After overnight incubation at 37°C, viable cells were counted as colony-forming units/milliliter (CFU/ml). The viable cell count of *S. aureus* was reduced to $1/10^3$ CFU/ml within 15 min of treatment with Burow's solution, and all bacterial cells were killed within 30 min of treatment. The viable cell count of *Pseudomonas aeruginosa* remained unchanged after 5 min of treatment, but was reduced to $1/10^3$ CFU/ml 40 min after treatment and to $1/10^7$ CFU/ml 60 min after treatment

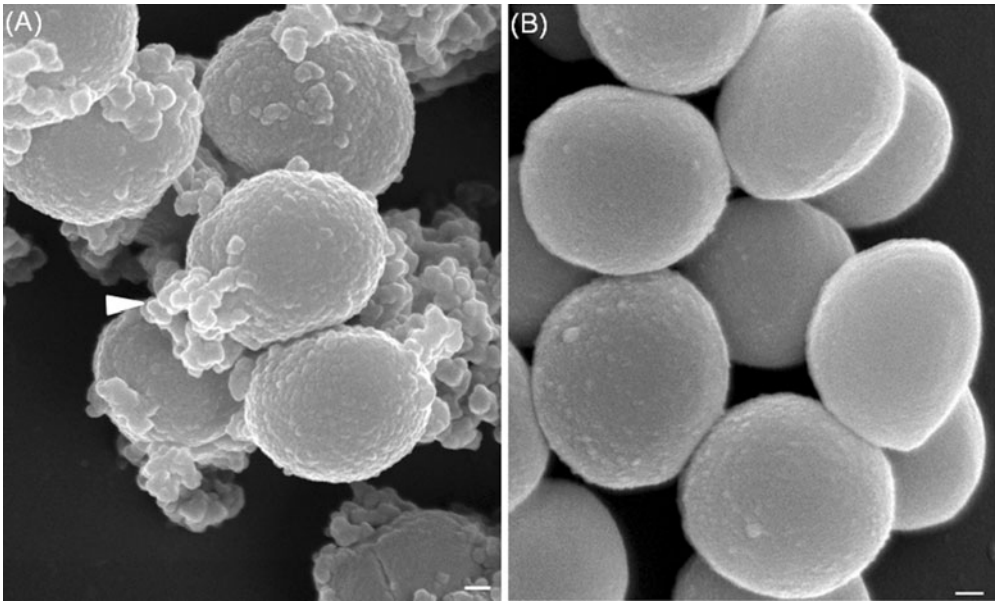

Fig. 2. Scanning electron microscopy observations of *Staphylococcus aureus* treated with Burow's solution for 15 min. Bleb-like structures and debris (*arrowhead*) on the cell surface were more apparent in the treated cells (**A**) compared with untreated cells (**B**). *Bars* 100 nm

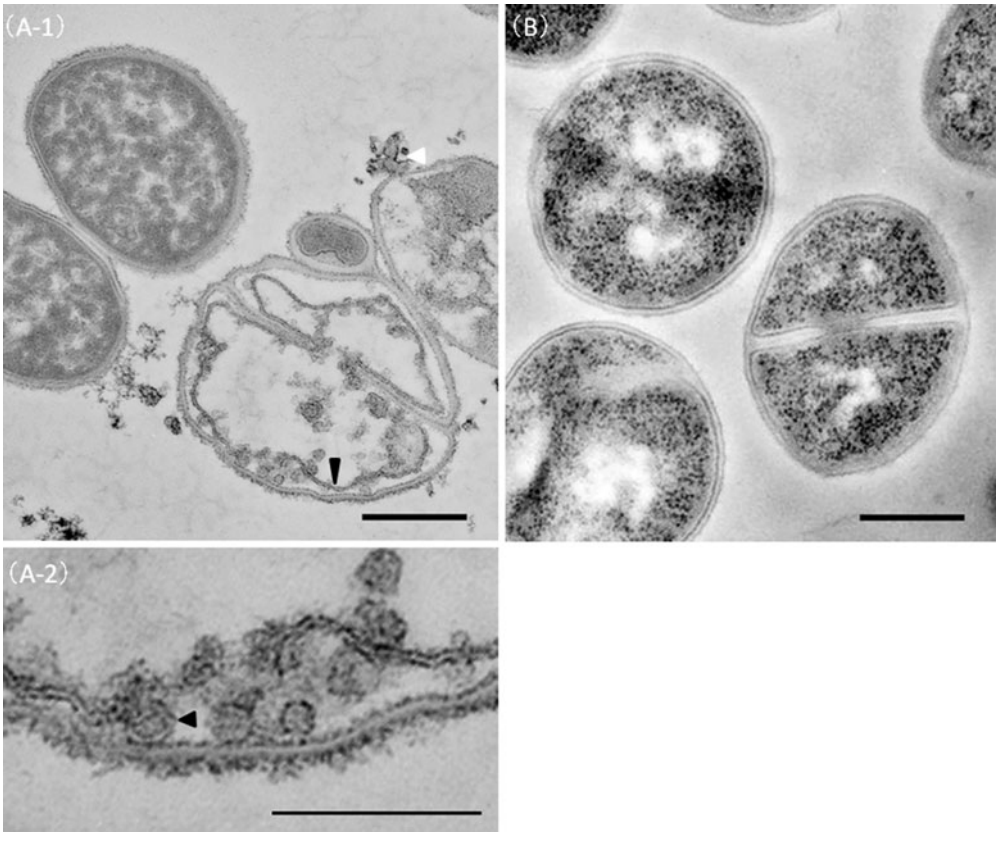

Fig. 3. Transmission electron microscopy observations of *Staphylococcus aureus* treated with Burow's solution for 15 min. Treated cells (**A**) exhibited undulating deformations of the cell wall (**A-1**), extracellular leakage of cell content (*white arrowhead*), and diluted cytoplasm with detachment of the cytoplasm from the cell membrane (*black arrowheads*), as compared with untreated cells (**B**). The peptidoglycan layer did not exhibit any striking morphological changes (**A-2**). *Bars* 0.3 μm

aeruginosa, with a more pronounced effect apparent against *S. aureus* compared with *P. aeruginosa* at the same concentration.

Ultrastructural examination of bacterial cells treated with Burow's solution

The SEM observation of *S. aureus* after a 15-min treatment with Burow's solution (Fig. 2A) revealed a rougher outer layer and blebbing on the cell surface, as opposed to a smooth outer layer for untreated cells (Fig. 2B). Characteristic morphological alterations caused by Burow's solution are shown in Fig. 3A; control cells are shown in Fig. 3B. Exposure for 15 min in Burow's solution led to breakage of the cell wall in *S. aureus*, exhibiting an undulating surface (Fig. 3A-1). The cytoplasmic membrane was clearly damaged, although the peptidoglycan layer did not exhibit any striking morphological changes (Fig. 3A-2). When the damaged cell was observed in detail, the cytoplasmic

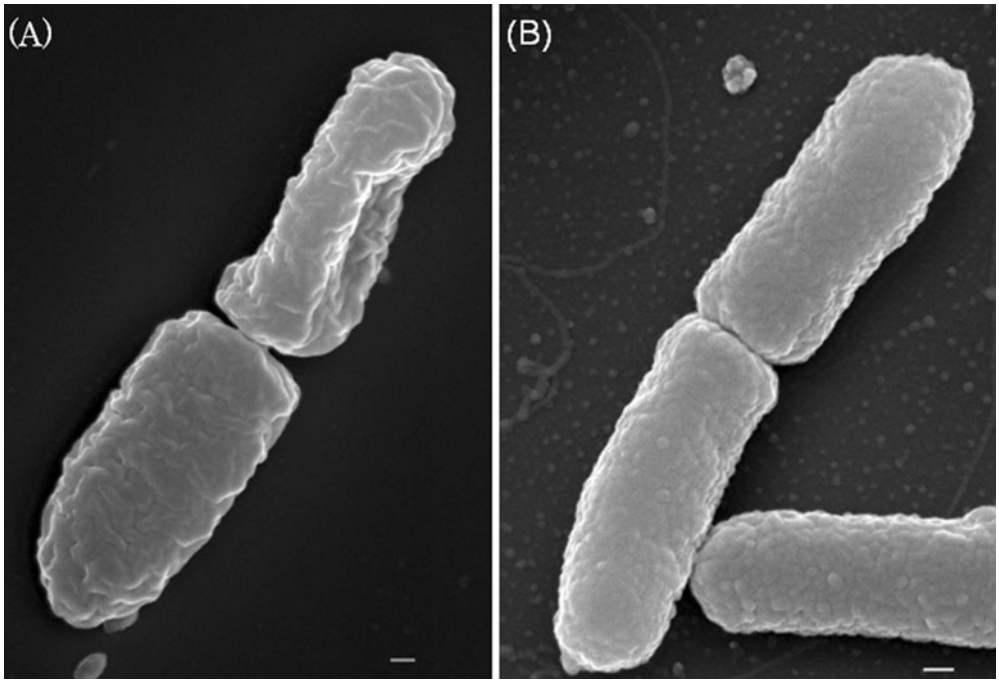

Fig. 4. Scanning electron microscopy observations of *Pseudomonas aeruginosa* treated with Burow's solution for 15 min. Treated cells (**A**) showed undulating deformation and partial collapse of the cell surface as compared with untreated cells (**B**). *Bars* 100 nm

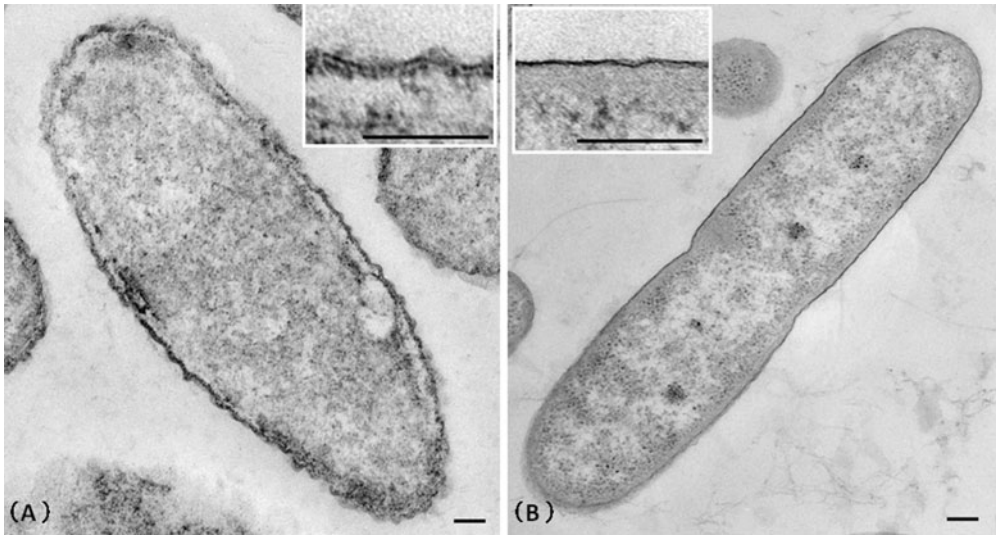

Fig. 5. Scanning electron microscopy observations of *Pseudomonas aeruginosa* treated with Burow's solution for 15 min. Treated cells (**A**) showed undulating deformations of the cell wall and cytoplasmic membrane, with a diluted cytoplasm, compared with untreated cells (**B**). *Bars* 100 nm

membrane composed of the lipid bilayer could be encircled before the formation of small vesicles (Fig. 3A-1, A-2). The damaged cytoplasmic membrane resulted in a vacuolated space appearing between the cytoplasmic membrane and the peptidoglycan layer. The destruction of the cell wall was followed by the formation of bleb-like structures on the surface (Fig. 3A-1) in conjunction with the appearance of a diluted cytoplasm (Fig. 3A-1). The bleb-like structure shown by TEM observation was equivalent to the debris observed on the surface using SEM.

SEM observations of *P. aeruginosa* cells revealed more apparent undulating deformation of the bacterial cell surface in treated cells (Fig. 4A) compared with normal untreated cells (Fig. 4B). SEM examination of *P. aeruginosa* cells revealed partial collapse of the bacterial cell surface following treatment with Burow's solution. TEM observations revealed undulating deformations on the bacterial cell wall and cytoplasm, with a diluted cytoplasm more apparent in treated cells (Fig. 5A) compared with normal untreated cells (Fig. 5B). The damaged cell wall of *P. aeruginosa* when observed in detail appeared to be separated from the cytoplasmic membrane (Fig. 5A), although these structures were not apparent in the untreated cells (Fig. 5B).

Discussion

Antibiotic ear drops have commonly been used for the treatment of chronic otitis media and otitis externa.[12]

However, antibiotic-resistant bacteria are increasingly isolated from ears affected by these diseases,[13] often making treatment difficult. Burow's solution has been shown to be effective in treating these conditions even in the presence of certain antibiotic-resistant bacteria and is currently attracting a large amount of attention by otorhinolaryngologists.

We previously demonstrated the antimicrobial effects of Burow's solution against clinically isolated antibiotic-resistant strains of *S. aureus* and *P. aeruginosa*, as well as against antibiotic-sensitive strains, using the disc method.[14] In the present study, we quantitatively examined the antimicrobial effects of Burow's solution against the same bacterial species using viable cell counting. Treatment with Burow's solution resulted in the death of *S. aureus* cells within 30 min and reduced the viable count of *P. aeruginosa* to about $1/10^7$ CFU/ml within 60 min, demonstrating that the solution exerts its antimicrobial effects against both bacterial species within a shorter time frame than the antibiotics usually used (see Fig. 1). The foregoing findings also reveal stronger antimicrobial effects of Burow's solution against *S. aureus* compared with *P. aeruginosa*. This conclusion is supported by the observation that *P. aeruginosa* is generally resistant to antibiotics and antiseptics.[15] Given the difference in the minimal effective duration of treatment between both bacterial species suggested in the present study, additional studies are needed to examine the effect of Burow's solution against other bacterial species.

Although Burow's solution has been extensively studied in clinical settings, the mechanism of its antimicrobial activity has rarely been investigated. Thorp et al.[16] demonstrated a higher efficacy of Burow's solution compared to an acetic acid solution (1–3%). Kashiwamura et al.[17] presented the time-dependent changes of the bactericidal effects of Burow's solution against *S. aureus*, pneumococci, *Candida albicans*, and *Aspergillus* species. For *S. aureus*, our results demonstrated a stronger antimicrobial effect of Burow's solution than that observed in Kashiwamura's study; short-term treatment with the solution resulted in the death of all *S. aureus* bacterial cells. With regard to the bactericidal activity of Burow's solution, Thorp et al. speculated that the activity was mediated by the acidity of the solution and the effect of aluminum acetate.[18] Terayama et al.[5] also claimed that the activity was mediated by the strong acidity of the solution and the effect of aluminum acetate. Nevertheless, the mechanism of action of the solution remains unknown. We thus attempted to elucidate its mechanism of action using SEM and TEM. We were able to observe that a 15-min treatment with Burow's solution induced deformation of bacterial cells in both species (see Figs. 2–5). *S. aureus* cells underwent a process leading to bacteriolysis; a stronger effect of the solution against *S. aureus* was also confirmed by electron microscopy. It was speculated that the solution disrupts the cytoplasmic membrane of *S. aureus*, thereby causing leakage of the bacterial cell content and eventual bacteriolysis. The solution also disrupted the cell wall of *P. aeruginosa*; however, the effects may have been weaker than those against *S. aureus*. It was not clear why these different sensitivities occurred. In Fig. 4, the three layers (outer membrane, peptidoglycan, and inner membrane) were not apparent upon closer inspection at a higher magnification. Therefore, it was difficult to discuss the detailed mechanism of Burow's solution against *P. aeruginosa*. Further studies are needed to elucidate these mechanisms of action in detail.

The effectiveness of Burow's solution for the treatment of ear infections has been reestablished and it is frequently used in clinical practice to treat ear diseases. Nevertheless, studies examining its antimicrobial activity are extremely limited. In the present study, we confirmed the effectiveness of Burow's solution against *S. aureus* and *P. aeruginosa*, thereby supporting clinical use of the solution. We also determined the minimal effective duration of treatment with the solution in vitro as a reference to determine the optimal duration of use of the solution clinically. Our future task is to determine the minimal effective duration of treatment with the solution against other bacterial species and the duration of treatment required in clinical settings.

Conclusion

Burow's solution has antimicrobial effects against *S. aureus* and *P. aeruginosa*, causing alterations and damage to the cell wall.

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