



BRIEF REPORT

Diversity and antibiotic resistance profiles of bacteria isolated from the oral cavity of captive snakes

Stephany Young-Yusty ^a, Alexa Prescilla-Ledezma^{a,*}, Hilda Acosta-de Patiño^b

^a Departamento de Microbiología Humana, Facultad de Medicina, Universidad de Panamá, Panama City, Panama

^b Centro de Investigación e Información de Medicamentos y Tóxicos (CIIMET), Universidad de Panamá, Panama City, Panama

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KEYWORDS

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Oral microbiome;
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Abstract Venomous snake bites are a global public health issue, causing between 81 000 and 138 000 annual deaths and 400 000 permanent disabilities. This study investigated the oral bacterial diversity and antibiotic resistance profiles in captive *Viperidae* snakes. Oral swabs from 48 specimens across four species (*Porthidium lansbergii*, *Bothriechis nigroviridis*, *Cerrophidion sasai*, and *Bothrops asper*) were analyzed using culture-dependent methods. Bacterial isolation and identification using the VITEK 2 automated system revealed 41 strains from 12 genera, predominantly Gram-negative bacteria, including *Morganella morganii* (11 isolates) and *Providencia rettgeri* (10 isolates). Antibiotic susceptibility testing demonstrated significant resistance patterns, with 100% resistance to cephalothin and cefazolin in *M. morganii*, and emerging extended-spectrum β -lactamase (ESBL) production in *Enterobacter cloacae* and *Sphingomonas paucimobilis*. The comparative analysis showed that *P. lansbergii* exhibited the highest bacterial diversity. These findings highlight the complex oral microbiota of venomous snakes and underscore the need for evidence-based antibiotic strategies in snakebite management, particularly given the global rise in antimicrobial resistance.

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PALABRAS CLAVE

Familia *Viperidae*;
Microbioma oral;
Resistencia a
antibióticos;
Infecciones por
mordedura de
serpiente

Diversidad y perfiles de resistencia a antibióticos de bacterias aisladas de la cavidad oral de serpientes en cautiverio

Resumen Las mordeduras de serpientes venenosas son un problema de salud pública mundial, causando entre 81,000 y 138,000 muertes anuales y 400,000 discapacidades permanentes. Este estudio investigó la diversidad bacteriana oral y los perfiles de resistencia antibiótica en serpientes *Viperidae* mantenidas en cautiverio. Se analizaron hisopados orales de 48 especímenes de cuatro especies (*Porthidium lansbergii*, *Bothriechis nigroviridis*, *Cerrophidion sasai* y *Bothrops asper*) mediante métodos dependientes de cultivo. La identificación bacteriana mediante el

* Corresponding author.

E-mail address: alexa_prescilla@yahoo.es (A. Prescilla-Ledezma).

sistema automatizado VITEK 2 reveló 41 cepas de 12 géneros, predominantemente bacterias Gram-negativas, incluyendo *Morganella morganii* (11 aislamientos) y *Providencia rettgeri* (10 aislamientos). Las pruebas de susceptibilidad antibiótica demostraron patrones de resistencia significativos, con 100% de resistencia a cefalotina y cefazolina en *M. morganii*, y producción emergente de β -lactamasas de espectro extendido (BLEE) en *Enterobacter cloacae* y *Sphingomonas paucimobilis*. El análisis comparativo mostró que *P. lansbergii* exhibió la mayor diversidad bacteriana. Estos hallazgos resaltan la complejidad del microbiota oral en serpientes venenosas y subrayan la necesidad de estrategias antibióticas basadas en evidencia para el manejo de mordeduras, particularmente ante el aumento global de la resistencia antimicrobiana.

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The global burden of snakebites represents a significant public health challenge, particularly in tropical and subtropical regions. Every year, snakebites result in an estimated 1.2–5.5 million envenomations, with fatalities ranging from 81 000 to 138 000 individuals¹. The impact is disproportionately felt in rural areas of developing countries, where agricultural workers and their families face the highest risk due to frequent interactions with snake habitats. The socioeconomic consequences of snakebites extend beyond immediate health effects, often leading to long-term disability and loss of income for victims and their families¹. In Central America, the situation reflects global trends. The region faces significant challenges in snakebite management, including inadequate healthcare infrastructure, lack of trained personnel, and insufficient availability of antivenoms. For instance, Panama records over 2000 snakebite cases, with a 2–3% fatality rate. Between 2007 and 2021, snakebite incidence ranged from 34 to 65 cases per every 100 000 inhabitants per year⁸.

The oral microbiome of venomous snakes plays a crucial role in both snake ecology and the clinical implications of snakebites. This complex ecosystem harbors a diverse array of bacteria, including potentially pathogenic species, such as *Morganella morganii*, and members of the *Enterobacteriaceae* family². These bacteria can cause severe infections following snakebites, manifesting necrotizing fasciitis and abscess formation. The composition of the snake oral microbiome is influenced by various factors, including environmental conditions and dietary habits. This dynamic interplay between snakes and their environment underscores the ecological complexity of snake microbiomes and their potential implications for zoonotic infections in humans⁵.

A particularly concerning aspect of snake oral microbiomes is the prevalence of antibiotic-resistant bacteria. Studies have identified multiple bacterial strains that are resistant to common antibiotics such as amoxicillin, penicillin, and oxacillin. Studies on Taiwanese venomous snakes showed a significant proportion of antibiotic-resistant oral bacteria². The presence of antibiotic resistance genes (ARGs) in snake oral microbiomes reflects a broader trend observed in various animal species, potentially exacerbat-

ing the global issue of antimicrobial resistance. The transfer of resistance genes from snake oral bacteria to human pathogens is also a growing concern, as it could further complicate the treatment of snakebite-related infections and contribute to the broader challenge of antibiotic resistance¹³.

This study investigated the bacterial diversity present in the oral cavity of captive *Viperidae* snakes. The research project characterized bacterial cultures obtained from oral cavity swabs, identified aerobic bacteria using biochemical methods, and evaluated their antibiotic resistance and sensitivity profiles. This significance of this study lies in its potential to advance our understanding of snake microbiomes and their implications for human health, particularly in the context of snakebite treatment.

The present study analyzed a total of 48 venomous snake specimens maintained in captivity at the Serpentarium of the Center for Research and Information on Drugs and Toxins (CIIMET) at the Universidad de Panamá, comprising 21 *Porthidium lansbergii*, 8 *Bothriechis nigroviridis*, 15 *Cerrophidion sasai*, and 4 *Bothrops asper* individuals. For *B. asper*, CO₂ sedation was used due to their large size, while smaller species were manually restrained. Swab samples were immediately placed in Amies liquid ESwab® transport medium. Samples were cultured on Blood Agar, MacConkey Agar, and Chocolate Agar under aerobic conditions. After incubation, colonies were isolated and subjected to Gram staining. Isolated colonies of bacteria were then identified using the VITEK® 2 COMPACT automated system (bioMérieux). Bacterial suspensions were prepared to a 0.5 McFarland standard and inoculated into the appropriate VITEK® 2 cards for both identification and antimicrobial susceptibility testing. GN and GP cards were used for Gram-negative and Gram-positive bacteria identification, respectively, while AST-N401 and AST-P663 cards were used for antibiotic susceptibility testing. The VITEK® 2 system provided biochemical identification of bacteria based on various tests and determined antibiotic resistance profiles following Clinical and Laboratory Standards Institute (CLSI) guidelines.

A total of 41 bacterial strains belonging to 12 different genera were isolated and identified from 48 initial sam-

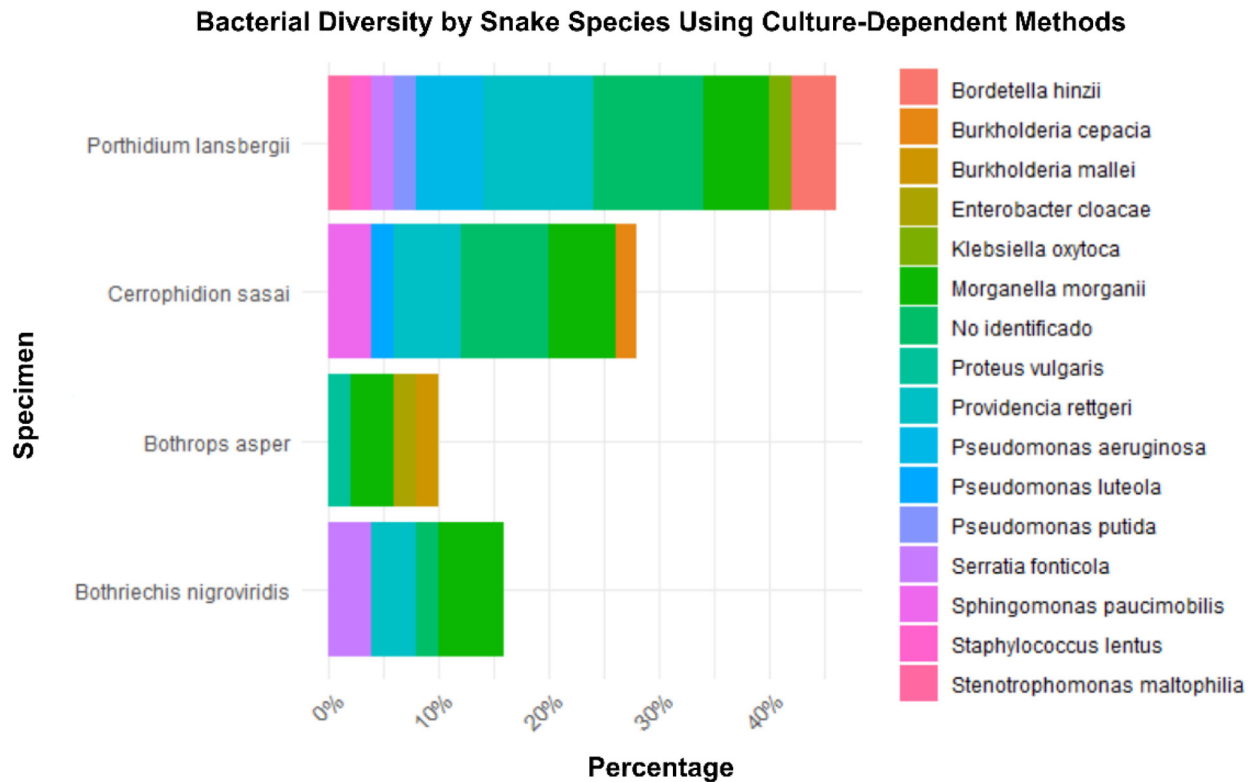


Figure 1 Bacterial diversity by snake species using culture-dependent methods. This bar plot compares the bacterial diversity isolated from the oral cavity and venom of four snake species. *Porthidium lansbergii* exhibits the highest diversity, followed by *Cerrophidion sasai*, while *Bothrops asper* and *Bothriechis nigroviridis* show lower diversity levels.

ples. The most frequently isolated species were *M. morganii* (11 isolates), *Providencia rettgeri* (10 isolates), and *Pseudomonas aeruginosa* and *Serratia fonticola* (3 isolates each). Gram-negative bacteria predominated in the oral cavities of the analyzed snakes. The comparison between snake species revealed interesting patterns in oral microbiota. *P. lansbergii* exhibited the highest bacterial diversity, with nine different species identified, while *B. nigroviridis* and *B. asper* showed lower diversity (Fig. 1). *M. morganii* was found in all snake species, while *P. rettgeri* was common in *B. nigroviridis*, *C. sasai*, and *P. lansbergii*. Some species-specific bacteria, such as *Proteus vulgaris* and *Enterobacter cloacae* in *B. asper*, and *Klebsiella oxytoca* and *Staphylococcus lentus* in *P. lansbergii*, were also identified.

P. lansbergii exhibited significantly higher bacterial diversity (Shannon index = 2.413, species richness = 12) compared to the other three species. *B. asper* showed the lowest diversity (Shannon index = 0.611, species richness = 2), representing a 295% difference in diversity between the highest and lowest species. The coefficient of variation for Shannon diversity was 49.2%, indicating substantial interspecies variation (Fig. 1).

Antibiotic susceptibility testing revealed variable resistance patterns among the isolated bacteria (Fig. 2). Notably, high resistance to cefalothin and cefazolin was observed in *M. morganii* and *P. rettgeri* isolates. Some *M. morganii* isolates demonstrated resistance to ertapenem, indicating potential carbapenemase production in these strains. Other strains, including a *Sphingomonas paucimobilis* isolate

and *E. cloacae* isolate, showed multidrug resistance profiles suggestive of extended-spectrum β -lactamase (ESBL) production. These findings highlight the diverse and potentially antibiotic-resistant bacterial communities present in the oral cavities of captive venomous snakes, which may have implications for snakebite treatment and management.

The selection of culture media, including Blood Agar, MacConkey Agar, and Chocolate Agar, was based on their widespread use in clinical microbiology and their ability to facilitate the isolation and identification of a wide range of medically relevant bacteria. Blood Agar, an enriched medium, allows for the growth of a variety of microorganisms, including both Gram-positive and Gram-negative bacteria, and facilitates the detection of hemolysis. MacConkey Agar, a selective and differential medium, is used for the isolation of Gram-negative bacteria, especially enterobacteria, and facilitates the differentiation of lactose-fermenting species. Chocolate Agar, an enriched medium containing additional growth factors, is used for the isolation of fastidious bacteria, such as *Haemophilus* and *Neisseria*.

Our results revealed a notable diversity of cultivable bacteria in the oral cavity of the studied venomous snakes. We successfully isolated and identified 41 bacterial strains belonging to 12 different genera. However, it is important to note that not all samples yielded bacterial growth on the media used, and some colonies could not be biochemically identified. This finding underscores the inherent limitations of culture-dependent methods, as not all bacteria present

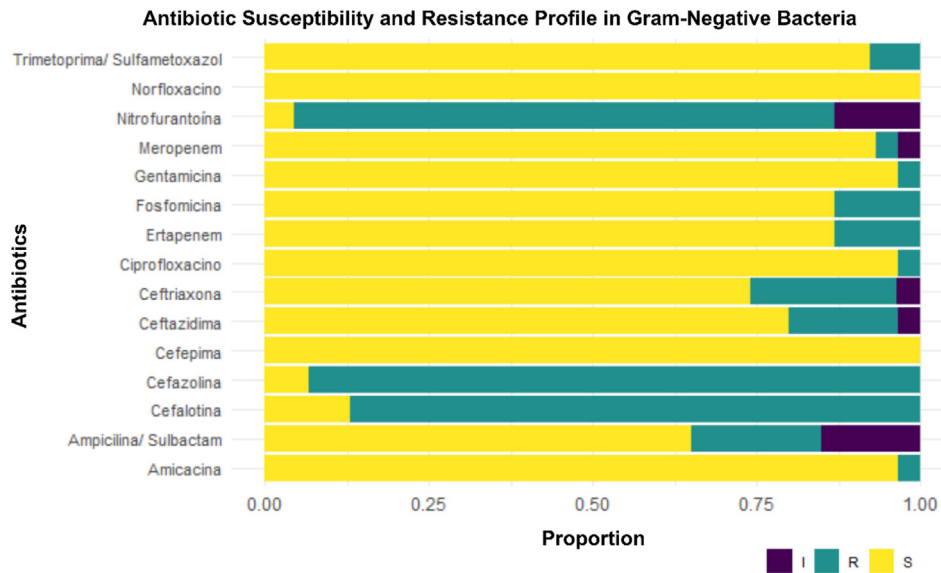


Figure 2 Antibiotic susceptibility and resistance profile in Gram-negative bacteria. This bar plot shows the proportion of Gram-negative bacteria classified as susceptible (S), intermediate (I), or resistant (R) to various antibiotics, highlighting differences in effectiveness.

in a complex ecosystem like the oral cavity are cultivable or identifiable using these techniques.

With regard to Gram-positive bacteria, the results revealed the identification of *S. lentus* in one sample. This finding suggests that Gram-negative bacteria might be fundamental components of the oral microbiota of snakes, regardless of geographical region. The observed differences in bacterial composition among the four studied snake species are particularly interesting. *P. lansbergii* exhibited the highest bacterial diversity, while *B. asper* and *B. nigroviridis* showed more limited diversity. These variations could be attributed to various factors, including differences in species-specific adaptations with respect to habitat and physiology. Additionally, they might reflect variations in intrinsic immunological defense mechanisms of each snake species.

The presence of *M. morganii* in all studied snake species is remarkable and consistent with its frequent isolation in previous studies. This bacterium has been associated with severe infections in humans following snakebites. In humans, *Morganella* can cause infections in various systems, including the bloodstream, urinary tract, and respiratory system, underscoring their clinical importance⁷. The genus *Morganella* comprises Gram-negative bacilli commonly found in water, soil, and the intestinal flora of various animals. This widespread presence underscores the potential for zoonotic transmission and the importance of monitoring antibiotic resistance in environmental isolates¹⁰.

P. rettgeri, the second most frequently isolated and identified bacterium in this study is considered an opportunistic pathogen. Bacteria of the genus *Providencia* can be found in various environments and organisms, with some species being part of the human intestinal microbiota. Among the species most frequently isolated in clinical contexts and responsible for urinary and nosocomial infections in humans are *P. alcalifaciens*, *P. rettgeri*, and *P. stuartii*¹⁵.

P. aeruginosa and *S. fonticola* exhibit Gram-negative bacilli morphology and inhabit various niches, such as soil, water, and associations with plants and animals. *P. aeruginosa* produces enzymes such as proteases and elastases, capable of degrading immunoregulatory proteins, including surfactants A and D, immunoglobulins, and antibacterial peptides¹¹. *S. fonticola*, on the other hand, has the ability to form biofilms, invade human corneal epithelial cells, cause keratitis, and produce gelatinase, elastase, and alkaline protease¹².

With respect to the antibiotic resistance profiles obtained in this study, we analyzed the antimicrobial resistance and sensitivity patterns of bacteria isolated from the oral cavity of snakes. The antibiogram is a key tool for evaluating the susceptibility or resistance of bacteria to specific antimicrobials. This analysis includes quantitative tests such as the determination of the minimum inhibitory concentration (MIC), which measures the lowest concentration of an antibiotic capable of inhibiting visible bacterial growth. The use of international standards such as those of the CLSI ensures that results are comparable between laboratories and useful for guiding clinical decisions. Furthermore, advances in automated techniques have allowed for faster and more precise results, optimizing the clinical management of patients.

M. morganii exhibited high resistance to cephalothin and cefazolin (100% of strains), which is consistent with its known ability to produce extended-spectrum β -lactamases (ESBL) or low-level carbapenemases. However, this species demonstrated sensitivity to antibiotics such as ceftazidime, ceftriaxone, ertapenem, and meropenem, suggesting that these agents could be effective in treating *M. morganii* infections. Previous studies have documented the association of this bacterium with nosocomial infections and its ability to develop resistance to multiple classes of antibiotics, highlighting its clinical importance⁹.

P. rettgeri showed universal resistance to cephalothin, cefazolin, and nitrofurantoin, but high sensitivity to ceftazidime, ceftriaxone, cefepime, and carbapenems, which aligns with previous results highlighting its role as an opportunistic pathogen in urinary and nosocomial infections⁶. Resistance to nitrofurantoin is a commonly observed pattern in *P. rettgeri*, limiting its therapeutic use. In the case of *S. fonticola*, resistance to cefazolin, ceftazidime, and ceftriaxone was observed, but sensitivity to cefepime, meropenem, and fluoroquinolones was noted. This is consistent with previous research that has reported variations in susceptibility profiles among different *Serratia* species¹⁴.

P. aeruginosa showed intrinsic resistance to cefazolin in all strains, which is a widely documented characteristic trait due to the natural impermeability of its outer membrane. However, variable sensitivity to meropenem (66.7% sensitive) and high sensitivity to ceftazidime, cefepime, and aminoglycosides were observed. This profile is consistent with studies highlighting the relative efficacy of carbapenems and aminoglycosides against *P. aeruginosa*⁴. Additionally, its ability to form biofilms could influence the observed variability in antimicrobial sensitivity. The isolation of *E. cloacae*, with a profile compatible with ESBL production, highlights its relevance as an emerging pathogen in nosocomial infections.

The observed resistance to third-generation cephalosporins underscores the need for continuous surveillance to mitigate the clinical impact of these resistant strains. The detection of ESBL or carbapenemase-producing strains in species such as *M. morganii*, *E. cloacae*, and *S. paucimobilis* reinforces the global concern about the dissemination of resistance genes among Gram-negative bacteria³. These enzymes not only confer resistance to β -lactams but also limit available therapeutic options.

The obtained results emphasize the importance of conducting specific follow-up tests to identify underlying molecular mechanisms, such as ESBL or carbapenemase-encoding genes (e.g., blaCTX-M or blaKPC), to guide more precise therapeutic decisions. Furthermore, continuous monitoring of the antimicrobial profile is crucial to prevent outbreaks associated with multidrug-resistant bacteria. The finding of *S. lentus* as the only Gram-positive bacteria isolated, represents a relevant contribution to the knowledge about snake-associated microbiota and its potential implications for public health. Recent studies have identified *S. lentus* in various contexts, such as animal infections and environmental contamination. A previous study reported the ability of some *S. lentus* strains to cause bacteremia in small animals, suggesting an underestimated pathogenic risk¹⁵. The absence of an antimicrobial susceptibility profile for this strain limits our understanding of its resistance or sensitivity to specific antimicrobial agents.

It is important to note that, although the Vitek 2 Compact system uses phenotypic methods based on susceptibility patterns to predict the presence of ESBL and carbapenemase producers, it does not genetically confirm these resistance mechanisms. Further molecular confirmation would be necessary to definitively validate these findings and to fully characterize all the resistance mechanisms.

Our results contribute to the growing body of knowledge on the oral microbiota of venomous snakes and its potential implications for snakebite management. The predominance

of Gram-negative bacteria, particularly *M. morganii* and *P. rettgeri*, in the oral cavities of the studied snakes aligns with previous research and suggests a consistent pattern across different geographical regions. This information is crucial for developing targeted treatment strategies for snakebite-related infections. The observed differences in bacterial composition among snake species highlight the complexity of snake oral microbiomes and underscore the need for species-specific considerations in snakebite management. The high bacterial diversity found in *P. lansbergii*, for instance, may have implications in the risk and severity of secondary infections following bites from this species.

It is important to note that the culture-dependent approach under aerobic conditions inherently excluded anaerobic bacteria, which can constitute a significant portion of oral microbiomes, as well as fastidious organisms requiring specialized growth conditions. Consequently, this may underestimate the true pathogenic potential and resistance mechanisms present in snake oral cavities that are relevant to snakebite management. Therefore, the actual bacterial diversity in these samples may be significantly underestimated due to these methodological constraints.

The antibiotic resistance profiles observed in this study, particularly the high resistance to certain antibiotics in *M. morganii* and *P. rettgeri*, raise concerns about the potential challenges in treating snakebite-related infections. The detection of ESBL or carbapenemase-producing strains further emphasizes the importance of careful antibiotic selection and the need for ongoing surveillance of antimicrobial resistance in snake-associated bacteria. These findings have important implications for both veterinary medicine and clinical therapy of venomous snakebites. They suggest that antibiotic treatment following snakebites should consider the potential presence of resistant Gram-negative bacteria. Furthermore, the identification of potentially pathogenic bacteria in snake oral cavities underscores the importance of prompt and appropriate wound care following snakebites to minimize the risk of secondary infections. Future research should focus on expanding our understanding of snake oral microbiomes across different species and geographical regions. The use of advanced molecular techniques, such as full-length 16S rRNA next-generation sequencing could provide a more comprehensive picture of the bacterial diversity in snake oral cavities. These approaches could help identify uncultivable bacteria and provide insights into the functional roles of different bacterial species within the oral microbiome of snakes.

ORCID ID

Alexa Prescilla Ledezma: 0000-0001-5324-6344

Hildauro Acosta: 0000-0001-8580-1402

Ethical disclosure

All procedures adhered to international standards for ethical animal research, including the ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals.

The study protocol (CEIBA-UP-032-2024) was reviewed and approved by the Research Ethics and Animal Welfare Committee (CEIBA) of the University of Panama.

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Conflicts of interest

The authors declare no conflicts of interest.

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