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Review

From Culture to Metagenomics: Evolution of Methodologies and Workflows in the Study of Oral Microbiota of Venomous Snakes

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Abstract

Venomous snakes constitute ecologically significant and medically relevant organisms due to the risks associated with their bites, which frequently result in complex secondary infections. The oral microbiota of these reptiles plays a crucial role in the pathogenesis of such infections; however, its diversity and clinical implications remain insufficiently characterized. This review synthesizes current knowledge on the oral microbiota of snakes, tracing the methodological evolution from traditional culture-dependent techniques to advanced culture-independent approaches, including next-generation sequencing and metagenomics. The analysis highlights the transformative impact of these technologies on bacterial diversity identification and antimicrobial resistance gene detection. Environmental factors, captivity conditions, and venom composition significantly influence microbial community structure and resistance profiles. These complex interactions are essential for improving clinical management of snakebite infections, informing empirical antibiotic therapy protocols, and guiding antivenom production strategies. Additionally, the potential of snake oral microbiota as a source of novel bioactive compounds represents an emerging area of bioprospecting research. This review demonstrates the necessity for integrative, multidisciplinary approaches to fully elucidate the ecological and biomedical significance of oral microbial communities in venomous snakes.

Keywords: snake oral microbiota; venomous snakes; culture-dependent methods; culture-independent methods; next-generation sequencing; metagenomics

1. Introduction

Venomous snakebites constitute a major global public health concern, with consequences ranging from immediate local effects to severe systemic complications [1]. According to the World Health Organization (WHO), snakebite envenomation is a global health priority, especially in areas with the highest incidence rates, primarily among rural populations in tropical and subtropical regions [2,3]. Recent estimates suggest that snakebite affects approximately 1.8 million persons each year, leading to approximately 94,000 fatalities every year [4,5]. This massive burden is further compounded by the complex interplay among ecological, socioeconomic, and human factors that affect the frequency of such events [5,6].

Beyond the immediate danger posed by venom, secondary infections post-envenomation, such as cellulitis and necrotizing fasciitis, represent significant complications that can exacerbate patient morbidity [7,8]. Although some snake venoms have antibacterial qualities, venomous snakes' oral microbiota can increase the risk of serious infections after tissue damage [9]. Studies reveal that bacterial infections after snakebite frequently require surgery and can cause permanent impairments for those who suffer from them [10,11]. These insights underline the urgency of addressing snakebite as a public health issue with multifaceted implications.

While snake venoms generally possess some antibacterial properties [12], the actual risks associated with the oral flora of snakes have gained attention in recent years. There is evidence that bacteriological profiles of snakebite wounds frequently contain both contaminants and possibly snake salivary microbiome-derived pathogens [9]. *Morganella morganii*, for example, is among the most frequent isolates from infected wounds, with the implication being that comprehensive microbiological evaluations are required in snakebite wound management [9].

There is a paradigm shift from culture-based to culture-independent approaches in the development of approaches to investigate the oral microbiota of venomous snakes. Traditional microbiological culture-based methods formed the basis of early research but were constrained by special growth requirements that can suppress the growth of certain microbiota [13]. The whole range of microbial life in snake mouths can now be explored using methods like next-generation sequencing (NGS) without the constraints of culturing because of paradigm shifts introduced by molecular biology innovations [9,12]. These sophisticated approaches provide a better understanding of the interconnection between snake venoms, oral bacteria, and possible post-envenomation complications.

The shift to culture-independent methodologies represents a broader trend in microbiological research aimed at unraveling complex microbial communities and their pathophysiological roles [14]. Through high-throughput sequencing techniques, researchers are now able to decode not only the composition of oral microbiota but also how these communities interact with snake venoms during envenomation events [12]. Furthermore, insights gained from this research may guide clinical practices for managing snakebite-related infections and lead to improved outcomes for victims.

The WHO's strategic campaign to mitigate morbidity and mortality from snakebites describes the rebranding of snakebite envenomation as a global health issue [14]. The overall strategy included within the WHO framework focuses on educational improvements among healthcare providers and community members. Increased education and training can result in quicker and more efficient reactions to snakebite, which will reduce the risks of infection and venom in the future [11,14].

In addition, the psychological effects of snakebite accidents cannot be downplayed; studies show victims developing long-term mental illnesses such as post-traumatic stress disorder and depression [4,15]. The psychological impact is usually aggravated by physical disabilities stemming from complications such as amputations or any other grave injuries [15]. Snakebite cases must thus be treated multimodally in consideration of psychological as well as physiological complications.

Following the development of culture-independent to culture-dependent methods for characterizing the oral microbiota of venomous snakes, this review aims to demonstrate the transformative power of next-generation sequencing technologies in expanding our knowledge of these intricate microbial communities. Culture-based methods, although old in creating early microbiological knowledge, have been hindered by their inability to capture all microbial diversity due to the high cultivation requirements and dominance of fast growers. The advent of molecular techniques, particularly 16S rRNA sequencing and metagenomics, has revolutionized our capacity for characterizing oral microbiota in its entirety, with culture-independent methods being capable of detecting much greater numbers of bacterial taxa than their culture-dependent counterparts.

2. Snakebite Complications and the Role of Oral Microbiota

Since there is a high rate of secondary infections due to bacteria entering the wound, the complications that arise from snakebite envenomation are a point of worry in treating the affected individuals. One of the most frequent outcomes following snakebite is secondary infection, particularly those caused by the genus *Bothrops*, with the proteolytic effect of venom creating an environment conducive to bacterial development [16]. Such infections, with a wide variety of pathogenic bacteria taking advantage of the tissue damage induced by the venom, can result in serious morbidity [9,17].

Snake venom also causes severe tissue damage on a pathophysiological level, and thus, the clinical presentation of envenoming becomes even more challenging. Most toxins that are brought by

the venom induce necrosis, inflammation, and an interference in normal tissue integrity that allows for colonization and infection by bacteria [18]. Interestingly, conditions like localized edema and increased vascular permeability contribute to the proliferation of bacterial pathogens that are usually inoculated by the snake's mouth [19]. Once tissue damage brought about by venom and snake oral flora are compounded together, it can cause severe localized infection like necrotizing fasciitis and cellulitis that necessitates immediate medical attention [19].

Numerous microbial species are implicated in secondary infections of snakebite wounds, according to epidemiological data on infections caused by snake oral flora. Research has indicated that infected bite wounds frequently harbor oral bacteria, including members of the *Enterobacter* and *Proteus* genera, *Morganella morganii*, and *Aeromonas hydrophila* [20,21]. Research demonstrating that these organisms can be isolated not only from the wound site but also directly from the snake's oral cavity highlights the significance of these bacteria and suggests a direct link between the microbial flora of the snake and infections that humans face after being envenomated [22]. These results highlight the vital need for efficient wound care procedures that take into account both the venom's immediate effects and the risk of infection from bacterial colonization.

In-depth analyses of infections caused by snakebite show that a wide variety of Gram-positive and Gram-negative species can be found in the wound microbiota. For example, studies of the oral microbiota of snakes have revealed the presence of several pathogenic bacteria, such as *Clostridium* and *Staphylococcus aureus*, as well as several other bacteria that are known to contribute to infections, including coagulase-negative staphylococci and *Pseudomonas aeruginosa* [22,23]. Because dietary choices can affect the microbial composition, additional classifications have revealed that the specific bacterial load in the oral cavity varies between snake species and their environments [24].

It is still difficult to monitor and control infection after snakebite attacks, particularly in the tropics, where resources could be scarce. Although advice differs with prevailing local vegetation, empirical antibiotic treatment is usually a component of present-day therapy in trying to cover a broad spectrum of likely infecting organisms [25,26]. Recent research made particular emphasis on the inefficacy of preemptive antibiotics against different local bacterial strains and their corresponding patterns of resistance [27]. This suggests the necessity of localized treatment guidelines and protocols, specifically those structured around regional snake populations' microbiome.

These results are definite proof that enhancing the clinical outcomes relies on understanding the constitution and function of the oral microbiota of snakes in envenomation cases. Understanding the nature of bacteria present can guide the use of the right antibiotics in improving the management of secondary infections [18,28]. In a bid to improve prevention and treatment practices and to ensure that patients are provided with timely and effective care so that venom toxicity as well as resultant infection is reduced, future studies targeting the establishment of the entire microbial profiles related to snakebite wounds shall be crucial.

3. Early Studies Using Culture-Dependent Methods

Initial research with culture-dependent methods has yielded immense information on microbial populations of snakebite infection. However, these methods have significant weaknesses with serious limitations that are critical to bias the accuracy and completeness of estimates of microbial diversity. One of the most significant limitations of traditional culture methods is perhaps a low resolution of microbial diversity (Figure 1). Typically, culture-based techniques isolate only a minuscule percentage, usually cited at 1-10%, of the total microbial variety that exists within an ecosystem, and in challenging ones like infected tissue following a snakebite, where several various bacterial groups coexist but few are identifiable using culturing [29].

The second fundamental deficiency of culture-based approaches lies in their inbuilt prejudice in favor of aerobic and fast-growing strains. Culture media and conditions employed are generally optimized for specific populations of bacteria that are unrepresentative of the real diversity present in nature. Most of the pathogenic bacteria that cause snakebite are anaerobes or slow growers, hence

easily overlooked under standard culture conditions [30]. This selectivity will lead to significant underrepresentation of such important pathogens as *Morganella morganii*, *Providencia sp.*, and *Aeromonas hydrophila*, which most frequently have been isolated from infected wounds and are causative agents of severe post-envenomation complications [29].

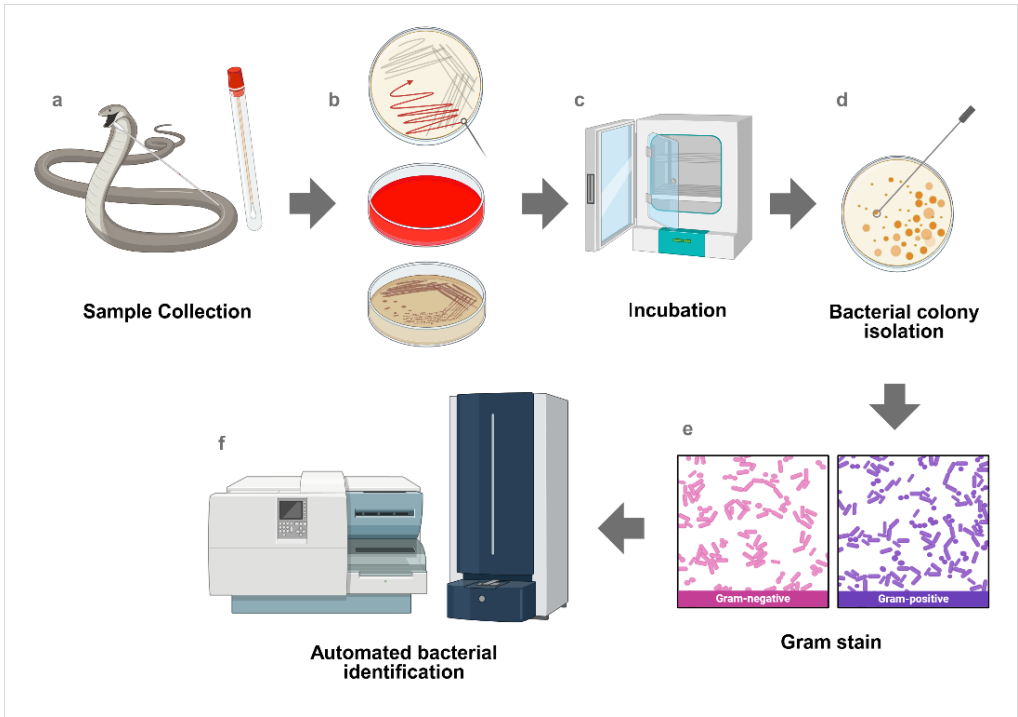


Figure 1. General workflow for bacterial identification using culture-dependent methods. (a) a biological sample is collected and (b) inoculated onto appropriate culture media, followed by (c) incubation under suitable conditions. After incubation, (d) isolated bacterial colonies are obtained and (e) subjected to Gram staining to differentiate between Gram-positive and Gram-negative bacteria. (f) Pure isolates are then identified using automated systems. This scheme is a general outline; specific steps may vary depending on the bacterial species’ nutritional and atmospheric requirements, as well as the laboratory’s identification equipment. Created in <https://BioRender.com>.

Detection of such genera as *Morganella* and *Aeromonas* holds significance because it can have the potential to directly inform empirical antibiotic therapy regimens so that healthcare workers may specifically target offending pathogens. Further, awareness of the existing bacterial community may also influence surgical decision-making and management of complications such as necrotizing fasciitis [31].

Regarding antivenom production, insights from microbial research stress the need for rigorous microbiological investigation. Since antivenoms are designed to counteract the toxic action of snake venoms, they must also be designed to compensate for the possibility of secondary bacterial infection that might complicate the clinical condition of patients. Bacterial population resistance genes could influence treatment efficacy and necessitate multi-target strategies in antivenom formulation [32,33]. By bridging the cultural gap between cultured and uncultured populations, researchers gain the capability to provide a stronger perspective on the pathophysiological world in which patients live, leading to better healthcare strategies and formulations.

4. Transition to Molecular and Culture-Independent Techniques

The transition from conventional culture-dependent techniques to culture-independent (molecular techniques) is a big leap in the study of microbial diversity, particularly in infectious complications due to snakebites. Polymerase chain reaction (PCR) and Sanger sequencing of 16S

rRNA gene fragments have been crucial in the discovery of molecular methods through which scientists can detect and identify bacteria that were previously unable to be cultivated in the laboratory setting. Sanger sequencing facilitated the amplification and sequencing of specific fragments of the 16S rRNA gene, which explained the structure of microbial communities in a variety of environments, like infected snakebite wounds [34,35]. The method has provided the specific taxa present in these samples and established a foundation for the understanding of bacterial infection dynamics.

However, both PCR and Sanger sequencing are constrained in that they do not sequence the entire 16S rRNA gene economically and relatively lack resolution for differentiating between closely related bacteria. Later, next-generation sequencing (NGS) technologies supplied remedies with the capacity to sequence numerous samples in parallel at high throughput. NGS has transformed microbial ecology through the ability to sequence longer amplicons and deeper coverage of microbial diversity, hence solving issues encountered with traditional sequencing methods [36].

Specifically noted is the use of 16S rRNA amplicon sequencing of the targeted hypervariable regions, such as V3–V4 (Figure 2). These are significant when it comes to taxonomic classification and are most widely used in microbiome studies. By applying a two-step PCR method and leveraging high-throughput sequencing technologies, researchers can amplify and sequence such regions to obtain fine-grained insights into microbial diversity among varying samples, e.g., snakebite wounds [34,37]. Sequencing data can be interpreted using alternative frameworks, namely amplicon sequence variants (ASVs) or operational taxonomic units (OTUs). ASVs, or unique sequences at single-nucleotide resolution, have better resolution for classification than OTUs, which cluster sequences by similarity and can lead to oversimplification or loss of some taxonomic information [38,39].

It has advantages in having increased higher taxonomic resolution, in which discrimination among closely related bacterial species that might be clumped together in classical culture-based studies can be made. For example, functional amplification and sequencing of the whole 16S rRNA gene can facilitate differentiation at the species level, providing more insight into the complexities in microbial infections ensuing from snakebites [40,41]. In addition, examination of the whole gene might provide leeway for debating functional niches of specific microbial taxa, and these can inform treatment protocols and clinical care strategies.

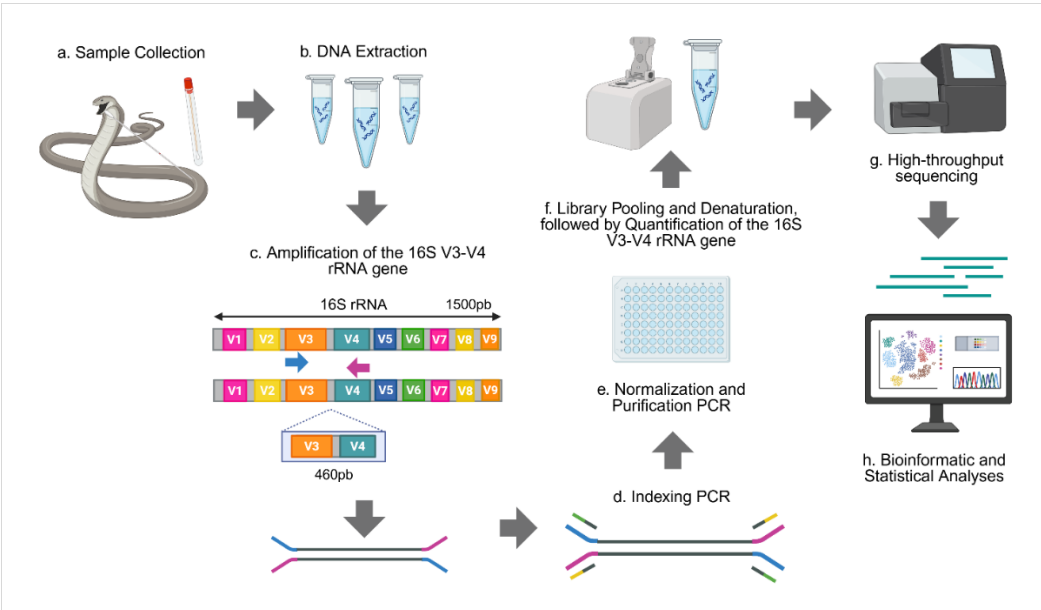


Figure 2. General workflow for bacterial identification using 16S rRNA amplicon sequencing targeting the V3–V4 hypervariable regions. (a) A biological sample is collected, and (b) DNA is extracted from the sample. Library preparations: (c) The V3–V4 regions of the 16S rRNA gene are amplified, followed by (d) indexing PCR to add adapter sequences. (e) Libraries are then normalized and purified, and (f) pooled and denatured, followed by quantification of the 16S V3–V4 amplicons. (g) The prepared libraries are subjected to high-throughput

sequencing. (h) Sequencing data are analyzed through bioinformatic and statistical approaches to determine microbial composition. This scheme is a general outline; specific steps may vary depending on the target microbiome, reagents, and sequencing platform used. Created in <https://BioRender.com>.

With such high taxonomic resolution, the capacity to detect rare and previously uncultivated species, and the comprehensive scale of NGS, microbial ecology in the context of snakebite disease is radically expanded. With such knowledge being of vital importance for clinical application, where familiarity with pathogenic organisms affected can guide antibiotic therapy and intervention strategies following envenoming, such a discovery has broad implications. Furthermore, microbiome analysis can support safer and more efficient antivenom treatment through the identification of how the bacterial infection could interact with venom components and impact patient results [42,43].

5. Current Advances: Full-Length 16S rRNA Gene Sequencing and Metagenomics

Recent studies involving next-generation sequencing techniques have identified bacterial communities of venomous snakes' oral cavities and the venoms more accurately. It has been particularly characterized by shotgun metagenomics and full-length 16S rRNA gene sequencing as powerful techniques that more clearly focus on the complex processes of such ecosystems.

The advances in the long-read sequencing technologies, such as Oxford Nanopore and Pacific Biosciences (PacBio), have enabled researchers to conduct full-length 16S rRNA gene sequencing. This would allow researchers to sequence the entire gene, achieving better resolution in microbial taxonomic identification. For example, in Lin et al.'s work in 2023, the authors used high-throughput sequencing to explore the oral microbiota of certain species of venomous snakes by sequencing and amplifying the full-length 16S rRNA gene, including hypervariable regions V1-V9. Rich microbial diversity was observed, which was responsible for the ecological roles of these bacteria within the snakes' environments [28].

It has been recently demonstrated that complete sequencing of the 16S rRNA gene is essential when it comes to the discrimination of closely related taxa, which may not be distinguishable when one considers only the V3-V4 regions typical of shorter-read strategies. The use of this technology allows for higher-scale ecological and phylogenetic assessments, hence expanding our understanding of the microbiome assemblies associated with various species of snakes.

Current research describes the dominant microbial phyla of the oral and fecal microbiota of snakes, such as Bacteroidetes, Proteobacteria, Firmicutes, and Fusobacteria [44,45]. The fecal microbiota of the Red Back Pine Root Snake (*Oligodon formosanus*) and Chinese Slug-Eating Snake (*Pareas chinensis*) have been specifically characterized, with research describing these dominant phyla and reporting significant differences in the composition of the microbiome between these two species, suggesting ecological and evolutionary adaptation [44]. Further investigation shows how ecological conditions may influence microbiome variation between species [45].

Shotgun metagenomics has been revolutionary, allowing not only for deep taxonomic profiling but also for functional characterization of microbial communities through the detection of genes implicated in specific metabolic pathways. The technique allows researchers to build a picture of the functional diversity of microbial populations, something that has been central to host-microbiota interaction studies. For instance, the important metabolic potential of oral microbes that would impact the overall health of the snake host has been described [46].

In addition, the application of shotgun metagenomics in studies concerning reptilian oral microbiomes has provided additional knowledge regarding pathogenic potential and resilience of the communities. Current studies have identified specific pathogens within such microbiotic communities, showing complexities linked to their ecological niches [6]. The studies are fundamental since they not only document the microbial diversity but also establish connections to potential health effects due to microbiome dysbiosis in snakes.

Moreover, recent advances have emphasized the application of full-length 16S rRNA gene sequencing, allowing for more accurate determination of phylogenetic relationships among bacterial populations. Investigation through high-throughput sequencing has revealed the phylogenetic diversity of Taiwanese venomous snake species' oral microbiota, allowing for subtle determination of the effect of evolutionary pressures on microbial populations. They emphasized the need for more advanced approaches to determine the complete taxonomic breadth of the oral microbiome [28].

These advanced methods hold colossal implications, particularly in light of emerging zoonotic pathogens. Through the discovery of the microbial interactions within the snake's oral cavity, researchers can develop an understanding of the potential reservoirs of pathogenic microbes, with the possibility of impacting not just snake health but also broader ecological processes. Snake oral cavity bacteria can serve as a reservoir for the spread of antibiotic resistance, demonstrating the public health significance of snake microbiome studies [47]

Furthermore, the exploration of dietary correlates with metagenomic research has identified how specific feeding habits determine oral microbiota structure in snakes [48]. This resolves fundamental questions concerning diet interaction with microbial ecology, where specific dietary specialization can enhance microbial taxa development that facilitates adaptability to their environments.

6. Microbiota-Host-Venom Interactions and Ecological Considerations

The complex interactions between the microbiota, host species, and venom in reptiles, and most notably in venomous snakes, provide a captivating window to study ecological interactions, evolutionary stress, and health impacts. The newer studies employing newer sequencing methods like full-length 16S rRNA gene sequencing and shotgun metagenomics have elucidated the effects of captivity, diet, and environmental parameters on microbial populations.

The composition of the gut microbiota is responsive to a variety of intrinsic and extrinsic factors, including captivity conditions, diet, and habitat quality. It has been found by research that captivity has a great impact on the gut microbiota of most species, leading to lower diversity and richness compared to the equivalent in the wild (Figure 3). For instance, research concluded that the intestinal bacterial community of the red-crowned crane was very dissimilar under captive and semi-free-range circumstances due to diet and habitat divergence [49]. This shows captivity is not only crucial for microbial diversity but also for host health and ecological adaptability.

Similarly, extreme variations in microbial diversity and function have been reported in captive alpine musk deer versus their free animals, demonstrating the extent to which captivity conditions and dietary supplies may fundamentally alter the gut microbiome [50].

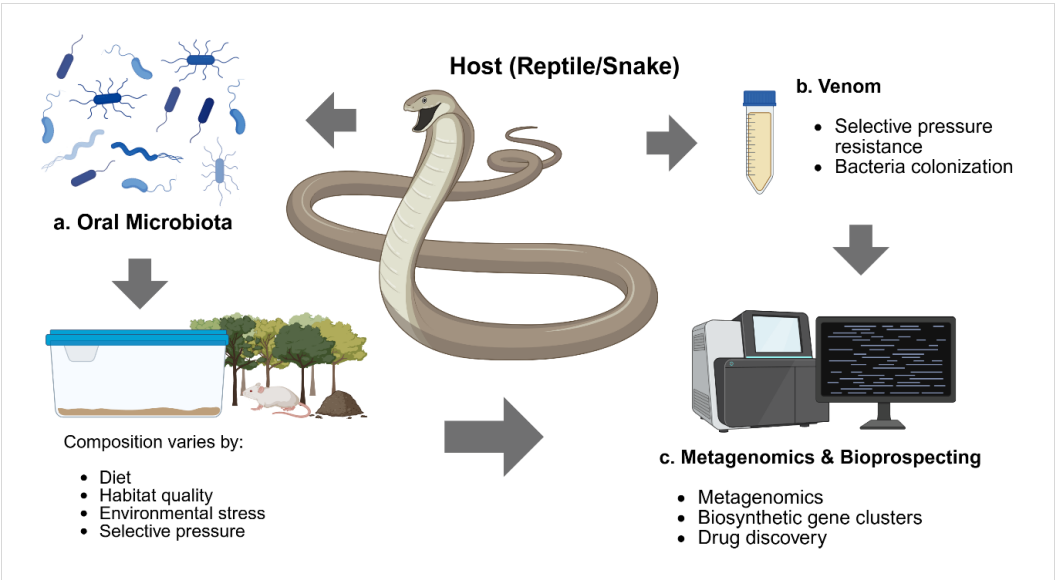


Figure 3. Conceptual framework illustrating the complex interactions between oral microbiota, host (reptile/snake), and venom in the context of metagenomics and bioprospecting research. (a) Oral microbiota consists of diverse bacterial communities whose composition varies according to multiple factors, including diet, habitat quality, environmental stress, and selective pressure from the host's biological systems. (b) Venom represents a unique ecological niche that exerts selective pressure on microbial communities, leading to bacterial colonization and potential development of resistance mechanisms. (c) Metagenomics and bioprospecting encompass the application of culture-independent molecular techniques, including next-generation sequencing, identification of biosynthetic gene clusters, and drug discovery approaches to explore the untapped potential of venom-associated microbiomes for novel antimicrobial compounds and therapeutic applications. Created in <https://BioRender.com>.

The host genus's role on gut microbiota has also been highlighted, demonstrating that there are unique microbial signatures to different primate species, as a result of diet and being captive [51]. The presence of venom in snakes may exert selective pressure upon co-occurring microbial communities. The action of venom on bacterial communities is particularly intriguing because venom components not only act upon prey but also upon the viability and even structure of microbes that colonize the snake's oral cavity.

Environmental stresses like venom profile and diet might have significant effects on microbial resistance profiles of snakes [52,53]. The dynamic interaction between venom and microbiota can lead to the creation of new resistant strains—either through natural selection or horizontal gene transfer—potentially enabling these bacteria to grow in extreme conditions. Furthermore, the identification of antimicrobial resistance genes in snake-associated microbiota has been highlighted as a priority area of research. Major studies have shed light on the idea that the recognition of microbial resistance mechanisms could reveal new bioactive compounds that will offer insights into the design of novel antibiotics or therapeutic agents [54]. As venom is toxic to or suppresses the majority of bacterial species, survivors may possess unique resistance characteristics or beneficial interactions with the host.

The information learned by shotgun metagenomics not only aids researchers in profiling microbial communities but also in finding functional genes that confer antimicrobial resistance or other bioactivities. These technologies enable one to interrogate microbial functions at higher resolution, capturing valuable genetic information on potential antimicrobial compounds that can be harnessed for use in human health [55].

For example, gut microbiome research in venomous snakes can identify new biosynthetic gene clusters that are responsible for the synthesis of new antimicrobial compounds. Such information can be very beneficial in pharmaceutical leads, especially with growing antibiotic resistance [56]. Venomous snake venoms and microbiota evolved together to form a biological niche rich with unexploited potential for bioprospecting [57].

7. Research Gaps and Future Directions

The research on oral microbiota across different species, particularly in tropical and subtropical ecosystems, indicates substantial knowledge gaps, including for venomous snakes and the ecological environments they inhabit. Although there have been improvements in methods used in microbiome studies, including shotgun metagenomics and full-length 16S rRNA gene sequencing, sampling and analysis of tropical species and areas continue to be underrepresented, contributing to a biased view of global microbiome diversity. The research that has been conducted focuses on reporting a substantial bias towards temperate ecosystems, with comparatively few studies aiming to target the microbial assemblages of tropical animals, such as reptiles like snakes [58–60]. The gap in extensive genomic profiling across tropical ecosystems continues, as research has also pointed out that many tropical freshwater environments continue to be inadequately described [58].

The underrepresentation of tropical species is a prominent gap in microbiome research since these environments support high levels of biodiversity and specialized ecological processes. For

instance, tropical environments such as caves or montane forests are often undersampled, potentially leading to significant microbiomes with important insights on microbial ecology and evolution at the global level to be overlooked [59,60]. This demographic and ecological sampling bias has consequences both for our understanding of tropical ecosystems themselves, as well as for the interpretation of microbial interactions and their impacts on host organisms within these regions.

Additionally, methodological discrepancies represent another obstacle to expanding our knowledge on reptilian microbiomes, particularly regarding standardization. It is critical that sampling, sequencing, and processing procedures are consistent because differences can result in significantly disparate findings. For instance, variation in DNA extraction procedures or library preparation steps can notably affect the observed microbial assemblages, making replication studies or comparison of results across studies challenging [61,62]. Protocol standardization helps generate reproducible information and promotes comparative research, leading to more expansive ecological conclusions.

There is an urgent need for integrated research connecting microbiota and venomomics to clinical outcomes. The potential relationship between the snake's oral microbiome and venom composition is extremely understudied. The oral microbiota, according to some researchers, could have implications for the efficacy, potency, and composition of snake venoms because the microbial community would metabolically interact with venom molecules or influence the ecological roles of venoms in prey immobilization or defense [63,64]. Clarification of these relationships would illuminate new avenues of research investigating ecological processes and implications for envenomation and therapeutic strategies in humans who are bitten by snakes.

It is argued that specific pathways are needed to bring together these areas of research, with a focus on a multidisciplinary melding of microbiology, zoology, and medicine. This is especially applicable within the tropics, where the range of venomous species tends to outstrip current medical understanding of their impact on human health [63]. In addition, as environmental shifts like climate change may have an impact on microbial interactions and species ranges, an understanding of these processes will be essential in forecasting ecological changes of the future and their implications for human health [65].

The promise of integrating venomomics and microbiota studies can be part of creating a comprehensive model of snake biology within their ecological niches. Investigation of the potential roles of the microbiome in enhancing or diminishing venom effectiveness can provide insight into how these creatures thrive and survive in their habitats [64]. Public health significance, particularly in snakebite-endemic regions where the condition remains largely untreated because of a lack of knowledge and means of effective antivenom strategies, should likewise be prioritized by scientists.

With these gaps in existing research, future studies must emphasize the characterization of tropical species and incorporate methodological standardizations to allow for multifaceted studies of the microbiomes of these ecosystems. The concerted effort to study ecological interactions using an integrated approach will elucidate the functions of microbiota in reptile biology and inform broader ecological and conservation objectives in tropical biodiverse environments.

8. Conclusions

The most important advancement in microbial ecology is the transition from culture-dependent to culture-independent methods. The conventional culture-dependent methods, though among the leaders during the initial periods of microbiological studies, are beset with deficiencies that make them grossly inadequate. The conventional methods can cultivate merely 1% of recoverable microbial diversity in the habitat from samples, thus resulting in the "great plate count anomaly."

Molecular techniques, 16S rRNA gene sequencing in particular, revolutionized microbial ecology with the potential of culture-independent examination of complex microbial communities. The 16S rRNA gene is a great molecular marker since it occurs in all prokaryotes, is evolutionarily conserved, yet contains hypervariable regions for species discrimination. The revolution was also fueled by the advent of next-generation sequencing (NGS) technologies that provided unprecedented

analysis depth and throughput. NGS platforms enabled researchers to sequence millions of bacterial 16S rRNA genes in parallel and reveal the true diversity and complexity of microbial communities.

The most recent quantum leap has been shotgun metagenomics, which sequences all the genomic material in an untargeted sample. This not only provides a comprehensive taxonomic profile but also characterizes the functional genetic potential of microbial communities, such as antibiotic resistance genes, virulence factors, and metabolic pathways. Application of molecular ecology techniques to characterize snake oral microbiota is now a fundamental component of modern snakebite treatment. Secondary bacterial infection is one of the most serious complications of envenoming following snakebite.

The therapeutic significance of oral microbiota profiling by deep oral microbiota profiling directly addresses therapeutic selection and patient outcome. Establishment of region-specific antibiotic regimens based on molecular profiling data is one of the greatest advancements in the treatment of snakebite. It has been found through studies that Gram-negative bacteria in the oral fauna of snakes have evolved reduced susceptibility to first- and second-generation cephalosporins.

The shift from classical microbiology to molecular ecology has transformed our understanding of snakebite envenomation's microbial communities. Culture-independent techniques have revealed the true complexity and diversity of oral snake microbiota, permitting evidence-based therapy for secondary infection.

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Abbreviations

The following abbreviations are used in this manuscript:

WHO	World Health Organization
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
rRNA	ribosomal Ribonucleic Acid
ASVs	Amplicon Sequence Variants
OTUs	Operational Taxonomic Units
PacBio	Pacific Biosciences

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