Review Article

Core and characteristic oral microorganisms in the giant pandas (*Ailuropoda melanoleuca*) suggested plenty of antibiotic resistance prevalence: A mini review

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Abstract

The giant panda (*Ailuropoda melanoleuca*) is a world-recognized rare wildlife endemic to China. Oral microorganisms may cause dental and other systemic diseases. Additionally, antibiotic resistance in this cute mammal may spread widely to travelers. In this mini review, we illustrated the oral core microorganisms of the giant pandas and their characteristics, as well as their prevalence of antibiotic resistance genes. Although there is limited availability in panda sample collection, researchers have made great efforts to find the characteristics of the giant pandas' oral microbiota, including *Enterococcus faecium*, *Enterococcus faecalis*, and Staphylococci, with antibiotic resistance genes *optrA*, *VanA*, *tetC* and so on. Our review suggested plenty of antibiotic resistance prevalence in the giant pandas. Therefore, it is essential to strengthen the monitoring of antibiotic resistance in giant pandas' oral microorganisms.

Keywords: antibiotic resistance, antibiotic resistance gene, giant pandas, oral microorganisms

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Introduction

The giant panda is an endangered mammal living in southwest China (Ma *et al*., 2017). It has gained worldwide recognition for its bamboo-specific dietary habits and adorable appearance (Yilmaz *et al*., 2015). The specific bamboo dietary habits significantly increase the chances of dental damage and dental diseases such as worn, chipped, and malformed teeth for the giant pandas (Ma *et al*., 2022). Additionally, unlike domesticated animals, giant pandas are difficult to manage in terms of dental care, which will increase calculus, defects and other oral diseases. On the other hand, the teeth of giant pandas play a crucial role in their food intake and digestion. Dental diseases or injuries can lead to a decline or loss of dental function, affecting their ability to eat and digest food, which may result in long-term starvation, systemic malnutrition, reduced body resistance, even the development of digestive system and other systemic diseases, ultimately leading to organ failure and death (Long *et al*., 2025). Therefore, dental health is an important factor that affects the overall well-being, life expectancy, and population size of giant pandas (Liu *et al*., 2023).

There is a close relationship between oral microorganisms and dental diseases. The presence of oral microorganisms can contribute to dental caries, periodontal diseases, and even other systemic diseases through synergistic or cooperative interactions (Singhal *et al*., 2011). Studies have shown that the unique characteristics of giant pandas' diet often led to malocclusion and the proliferation of oral microorganisms, resulting in a higher incidence of oral and gastrointestinal diseases, which can impact their growth, development, and even cause mortality (Jin *et al*., 2015). The oral cavity of giant pandas provides a suitable environment for the growth of various microorganisms due to its moisture, warmth, and the presence of salivary proteins, glycoproteins, and gingival sulcus fluid (Kilian *et al*., 2016; Solbiati and Frias-Lopez, 2018). These oral microorganisms can influence the balance between health and disease locally and systemically, and their interactions with the host play a significant role in the body's defense against external threats (Zarco *et al*., 2012). However, imbalances in the microbial community may occur when normally harmless microorganisms multiply under specific conditions. (Sampaio-Maia *et al*., 2016). Furthermore, oral microbial communities are closely associated with systemic diseases such as oral tumors, diabetes, rheumatoid arthritis, cardiovascular diseases, and preterm birth. Therefore, the structure of the oral microbial community can serve as an important indicator for monitoring oral and systemic health (Chen and Gong, 2023). The oral health status is a critical factor that affects the fitness, longevity, and population size of giant pandas. The literature included in this study was published from 2001 to 2024, encompassing more than two decades of research in this area of giant panda oral microbiology. In this review, we illustrated the oral core microorganisms of giant pandas and their characteristics, as well as the prevalence of antibiotic resistance genes. To provide some references for the oral health and of giant pandas.

Oral core and characteristics microorganisms of giant pandas

Currently, the interest of most researchers on giant pandas is focused on the microbial diversity of the intestinal tract and skin (Yang *et al*., 2018; Ma *et al*., 2021). More noteworthy is that the oral cavity structure and dietary characteristics of the giant panda have created its unique oral microbiota. The giant panda feeds on bamboo, which is a low-nutrient and high-fiber food. To reach its own energy requirements, the giant panda must eat all day up to 14 h (Jin *et al*., 2011). This feeding habit undoubtedly greatly increases the risk of tooth damage and dental diseases (Ma *et al*., 2022). The high caries rate of giant panda tooth fossils is related to the morphological structure and dietary habits of its teeth (Jiangzuo *et al*., 2024). Previous studies on the microbial community of the giant panda's oral cavity have primarily utilized traditional isolation and culture methods. For example, Feng *et al*. (2019) isolated and cultured bacteria from the oral cavity of captive giant pandas and identified the dominant bacterial groups as *Streptococcus* spp. and *Staphylococcus* spp. Shurong (2001) isolated *Micrococcus* spp. and *Aggregatobacterium* spp. from the secretion of infected oral cavities in panda cubs. Zhong *et al*. (2021) found *Enterococcus* spp. were the dominant genera in the oral cavity of giant pandas.

In order to better define the oral core and characteristic microbial classes and the association of microorganisms with oral health in giant pandas, this section will systematically sort out the composition and characteristics of microorganisms present in the giant panda's oral cavity at the phylum and genus levels by means of high-throughput sequencing studies. With the development of next generation sequencing technology, in a recent study conducted in 2023 (Wang *et al*., 2023). The oral microorganisms of giant pandas were classified as mainly Proteobacteria (70.4%) and Cyanobacteria (13.4%) at the phylum level. At the genus level, the main species identified were *Actinobacillus* (10.5%), *Lautropia* (5.4%), *Pseudomonas* (3.9%), and *Moraxella* (3.8%). The study also revealed the presence of pathogenic bacteria such as *Streptococcus*, *Actinomyces*, *Porphyromonas*, *Staphylococcus*, and other caries-related bacteria in the oral cavity of captive giant pandas. Therefore, the dominant bacteria in the oral cavity of giant pandas belong to the phylum Thick-walled Bacteria, Ascomycetes, Actinobacteria, and Mycobacteria at the phylum level. These bacteria act as the "core microbiome" in the oral microecological environment of giant pandas (Zaura *et al*., 2009), which is important for maintaining the balance of the oral microecological environment.

The abundance of bacteria in the salivary microbiota has been found to be significantly correlated with oral health status. In a 2019 study (Feng, 2019), the total number of oral bacterial isolates from adult and juvenile giant pandas was similar. However, some bacteria, including *Escherichia* spp., *Streptococcus* spp., *Pseudomonas* spp., *Fusobacterium* spp., and *Rothschild* spp., were more frequently found in juvenile giant pandas compared to adults. This difference in bacterial composition may be related to the susceptibility of juvenile giant pandas to diarrhea. *Staphylococcus* spp. were the most abundant isolates in the oral cavity of both juvenile and adult giant pandas, indicating that *Staphylococcus* spp. dominates the oral bacteria of healthy giant pandas. Common bacteria, such as *S. fleurettii*, *S. sciuri*, *S. equorum*, *S. lentus*, and *S. saprophyticus*, were found in both juvenile and adult giant pandas, but the isolation rates varied to some extent with age. This variation may be linked to differences in diet and living environments. Adult giant pandas live in separate hutches and have rotating outdoor activities, while juvenile giant pandas live in groups and have simultaneous outdoor access.

In contrast, a study in 2023 (Wang *et al*., 2023) found that the structure of oral microorganisms in subadult giant pandas was similar to that of older pandas. This similarity may be attributed to the fact that the sampled giant pandas lived in similar captive environments. The subadult pandas in the study were all around 3 years old and had completed the transition from a high-protein diet (e.g., breast milk or cow's milk) to a high-fiber diet dominated by bamboo and other fiber-rich foods. Their dietary structure resembled that of older pandas. The similarities in environment and dietary conditions may have contributed to the similarities in oral microorganisms (Shaw *et al*., 2017; Li *et al*., 2018).

Comparison of the oral core microorganisms of giant pandas with other species

The structure of oral microorganisms varies among different species, leading to differences in susceptibility to oral diseases. However, there are also similarities in the oral microbial structure among different species due to inevitable interactions. The dynamics and involvement of oral microorganisms in health and a variety of diseases have been studied extensively (Tanner *et al*., 2016; Santibáñez *et al*., 2021). However, in comparison with the gut microbiota, little is known about the diversity and evolution of oral microorganisms across mammals, as previous studies focused primarily on domestic animals and other primates (Li *et al*., 2013; Ozga *et al*., 2019; Ruparell *et al*., 2020a). A major factor is the difficulty of oral sample collection from wild animals (especially large mammals and carnivores), as compared to opportunistic fecal sampling. In a previous study, regarding the oral microorganisms of carnivores, research on wolves has revealed that the main microbial flora was not significantly different from that of herbivores through comparison (Podar *et al*., 2024). Further comparison found that, in the human oral cavity, the dominant phyla were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria (Chen *et al*., 2017). Research illustrated that healthy humans, companion animals, monkeys, rabbits and rats had similarities in their oral microorganisms. The oral microorganisms of humans, pigs, dogs, monkeys, rabbits and rats were dominated by *Actinobacillus*, *Bacteroides*, *Proteobacteria*, and *Firmicutes* (Gu *et al*., 2016). For the giant panda, five phyla had relative abundances higher than 1% in giant panda cubs' oral microbiota: Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Fusobacteria (Liu *et al*., 2023). These phyla have also been detected in oral microbiota studies of both humans and animals (Jiang *et al*., 2013; Willis and Gabaldón, 2020). Comparing the relative abundances at the phylum level, the oral microbiota of giant panda cubs is more similar to that of cats (Sturgeon *et al*., 2014) and cattle (Borsanelli *et al*., 2018), followed by kangaroo infants (Chhour *et al*., 2010), swine (Kernaghan *et al*., 2012), and then humans, chimpanzees, and bonobos. The most significant differences are observed in marine mammals (Sturgeon *et al*., 2013; Bik *et al*., 2016). Despite the similarity in tooth structure between giant pandas and canines (Weng *et al*., 2016; Coli *et al*., 2023), their oral microbiological composition is not the same. Several studies have shown that the oral microbiota in canines is dominated by bacteria from 14 different phyla, with Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, and Spirochaetes being the most detected types (Davis, 2016; Isaiah *et al*., 2017; Bell *et al*., 2020; Ruparell *et al*., 2020b; Wallis *et al*., 2021). However, the results of different studies varied to some extent.

Since the variety of daily diets, is different from similar detected existing strains, the bacterial community structure in the oral cavity of giant pandas is significantly different from that of humans. The predominant flora of the human oral cavity is Firmicutes, accounting for 23.2% of the total (Chen *et al*., 2017), and the predominant flora of the giant panda oral cavity is Proteobacteria, accounting for 70.4% of the total (Wang *et al*., 2023). Interest, although giant pandas are herbivores, the oral microbial structure of giant pandas differs from that of cattle and rabbits. In cattle, the most prevalent taxa in oral microorganisms are *Pseudomonas*, *Burkholderia*, and *Actinobacteria* (Borsanelli *et al*., 2018). For rabbits, *Streptococcus* spp., *Staphylococcus* spp., and *Actinomyces* spp. are the most commonly cultured oral microorganisms (Flenghi *et al*., 2023). The unique structure of oral microorganisms in giant pandas may be related to their specialized bamboo diet. However, there are some shared microorganisms. In 2022, Ma *et al*. (2022) conducted genomic analysis of dental plaque from giant panda cubs and detected the genus Porphyromonas, which is also found in humans and canines (Urmi *et al*., 2021). Studies have shown that *Porphyromonas* can be transmitted between canines and humans (Bai *et al*., 2023). As the number of captive giant pandas increases and their contact with humans intensifies, there is a potential for *Porphyromonas* transmission among giant pandas, canines, and humans.

Antibiotic resistance in oral core microorganisms of giant pandas

Currently, the emergence of bacterial antibiotic resistance is extremely critical. As known around the world as cute ornamental wildlife, the giant panda is also at great risk of spreading antibiotic resistance genes, especially in oral microorganisms. According to research, among the microorganisms isolated from the oral cavity of giant pandas, different microorganisms carried verity of antibiotic resistance genes, resulting in different resistance patterns. Zhong *et al*. (2021)

analyzed the prevalence and characteristics of antimicrobial *Enterococcus faecalis* isolated from the oral cavity of captive giant pandas in China. The findings revealed that all isolates showed 100% multidrug resistance. *E. faecalis* isolates exhibited higher resistance percentages to gentamicin (48.1%), vancomycin (55.6%), linezolid (100%), and streptomycin (33.3%) compared to *E. faecium* isolates. The *E. faecium* isolates displayed strong resistance to various antibiotics but were sensitive to high concentrations of aminoglycosides. Resistance rates to vancomycin, linezolid, and nitrofurantoin were higher than those reported in similar studies conducted in China and other countries. A high proportion of isolates carried antibiotic resistance genes such as *vanA*, *cfr*, *optrA*, *tetC*, and *ermA* were illustrated.

Another study in 2023 (Liu *et al*., 2023) revealed that a total of eight antibiotic resistance genes were detected in 28 isolates of *E. faecium*. The positive detection rate of blaCTX-M was 29%. The detection rates of linezolid-resistant genes *cfr* and *optrA* were 54% and 29%, respectively. Aminoglycoside-resistant gene aac(6')-aph(2') was detected only in 1 strain. The positive detection rate of aph(2'')-Id was 54%. The detection rate of *vanA*, a vancomycin-resistant gene against glycopeptide antibiotics, was 50%. The detection rate of *ermA* gene was as high as 100%, while the detection rate of *ermB* gene was only 39%.

In the study mentioned above (Feng, 2019), 80 coagulase-negative Staphylococci (CoNS) were identified, with 39 being from juvenile giant pandas and 41 from adult giant pandas. More than half of the strains exhibited multidrug resistance, and the five-drug resistance rate was 6.25%. Juvenile giant panda *Staphylococcus* strains were mainly resistant to 2 types of antibiotics (28.21%), while adult giant panda *Staphylococcus* strains were primarily resistant to 3 types of antibiotics (31.71%). Vancomycin exhibited the highest resistance rate (68.75%), followed by azithromycin (51.25%), whereas aminoglycoside and tetracycline resistance remained below 20%. *Staphylococcus aureus* (Feng, 2019) has the highest isolation rate of *BlaZ* gene (41.25%), followed by *mecA* (40.00%), *cfr* (38.75%), and *VanB* (25.00%).

These studies indicate that the types of antibiotic resistance vary among captive populations of giant pandas and may be related to factors such as different veterinary clinical antimicrobial drug usage practices at various sites, different types of bacterial diseases prevalent in different captive populations of giant pandas, and varying inputs of exogenous resistance genes. Additionally, antibiotic resistance can vary among individuals of different ages, emphasizing the need to consider age in the clinical use of drugs. The study also revealed that antibiotic resistance genes (ARGs) might spread from bamboo to the intestinal flora of giant pandas and further disseminate through feces (Yan *et al*., 2023). Therefore, there is a possibility that ARGs could be transmitted from bamboo to the oral cavity of giant pandas, forming an oral-gut-feces transmission system, where the relevance of drug resistance gene transmission is worthy of further research. Recent investigations on fecal-derived resistance in giant pandas have revealed high levels of multidrug-resistant bacteria. For example, studies have shown that *Escherichia coli* and *Salmonella* isolated from giant pandas exhibited varying degrees of resistance to 17 antibiotics, with tetracycline resistance reaching a maximum of 35.22% and amoxicillin resistance reaching a maximum of 42.55% in *Salmonella* (Su *et al*., 2021). Comparing the resistance of *E. coli* from giant pandas with that from other species, current studies suggest that *E. coli* resistance in giant pandas is higher than in other wild animals but lower than in food animals (Ghensi *et al*., 2020). This difference is hypothesized to be due to the higher resistance observed in wild animals and the lower resistance observed in food animals. It is assumed that giant pandas, as wildlife, have a restricted diet and strictly limited antibiotic use, resulting in lower resistance compared to food animals. However, giant pandas are more closely related to humans than other wildlife and have greater opportunities for antibiotic exposure, leading to higher bacterial resistance compared to other wild animals.

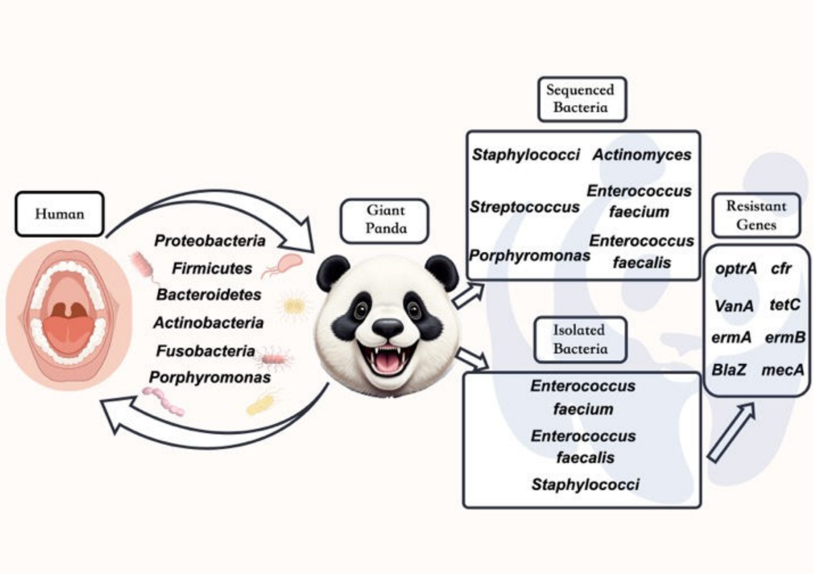

Figure 1 Graphical abstract.

Table 1 Oral core microorganisms of giant pandas in previous studies.

Bacteria genus	Year of study	Sample Sources	research target	Detection Methods	Key findings	reference
Streptococcus	2019	Oral secretions of captive giant pandas	Normal giant panda (juvenile, adult)	Traditional isolation and culture	More common in juvenile than in adult giant pandas	(Feng, 2019)
Staphylococcus	2019	Oral secretions of captive giant pandas	Normal giant panda (juvenile, adult)	Traditional isolation and culture	Juvenile, adult giant pandas have the highest isolation rate in the oral cavity	(Feng, 2019)
Micrococcus	2001	Giant panda cub infected with oral secretions	Infected giant panda (juvenile)	Traditional isolation and culture	Appearance in the oral cavity of diseased giant pandas	(Shurong, 2001)
Aggregatobacterium	2001	Giant panda cub infected with oral secretions	Infected giant panda (juvenile)	Traditional isolation and culture	Accompanying infection in juvenile giant pandas	(Shurong, 2001)
Enterococcus	2021	Oral secretions of captive giant pandas	Normal giant panda (adult), infected	Traditional isolation and culture	Oral dominant genera with significant multi-drug resistance	(Zhong *et al*., 2021)
Actinobacillus	2023	Captive giant panda oral samples (saliva, dental plaque)	Normal (subadult, elderly)	NGS	Co-occurrence with other pathogenic bacteria may contribute to the development of dental caries in giant pandas, with the highest genus level abundance (10.5%)	(Yang *et al*., 2018; Wang *et al*., 2023)
Lautropia	2023	Captive giant panda oral samples (saliva, dental plaque)	Normal (subadult, elderly)	NGS	Genus level abundance of 5.4% can be stabilized in healthy individuals	(Wang *et al*., 2023)
Pseudomonas	2023	Captive giant panda oral samples (saliva, dental plaque)	Normal giant panda, infected	NGS	More common in juvenile than in adult giant pandas.Genus level abundance of 3.9%	(Wang *et al*., 2023; Yang *et al*., 2023)
8	2023	Captive giant panda oral samples (saliva, dental plaque)	Normal (subadult, elderly)	NGS	The abundance was 3.8% at the genus level and was relatively stable in the oral microorganisms of giant pandas	(Wang *et al*., 2023)
Porphyromonas	2022	Giant panda cub with dental caries and plaque	Giant panda with dental caries (juvenile)	Whole Genome Sequencing (WGS)	Associated with dental caries, risk of cross-species transmission	(Ma *et al*., 2022; Bai *et al*., 2023; Wang *et al*., 2023)

Limitation and conclusion

Since the limited availability of giant pandas and the challenges in sample collection, there have been fewer studies on oral microorganisms in the giant pandas. The researchers had made great efforts to find the characteristics of the giant panda oral microbiota, including the core microbiome and drug resistance. Since the giant pandas are popular and attractive significantly, it increases the interaction between giant pandas and humans, facilitating the exchange of bacteria between the two species. Therefore, future research may focus on the following aspects: (1) Investigating the pathogenicity of antibiotic-resistant oral bacteria in giant pandas and exploring the correlation between antibiotic resistance and virulence. (2) Studying the giant panda-environment-human chain, including bacterial resistance and mobile elements, to understand the sources and transmission mechanisms of bacterial resistance in giant pandas. (3) Considering the integration of traditional Chinese medicine in the treatment of oral bacterial diseases in giant pandas. Additionally, while complete eradication of bacterial antibiotic resistance may not be feasible, reducing the selection pressure for resistance may help minimize the production and dissemination of antibiotic resistant genes. Therefore, it is essential to strengthen the monitoring of antibiotic resistance in giant panda oral microorganisms. Furthermore, implementing appropriate giant panda feeding and antibiotic resistance reducing management programs, especially for the care of juvenile giant pandas, may reduce the occurrence of oral and other systemic diseases.

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