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# Antimicrobial resistance and virulence factor gene profiles of *Enterococcus* spp. isolated from giant panda oral cavities

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## Abstract

**Introduction:** The objective of this study was to determine the prevalence and characteristics of antimicrobial-resistant *Enterococcus faecalis* and *E. faecium* isolated from the oral cavities of captive giant pandas in China. **Material and Methods:** The virulence-associated determinant and antimicrobial resistance genes were detected and antimicrobial susceptibility tests were performed on 54 strains of each bacterium. **Results:** All isolates showed 100% multidrug resistance. *E. faecalis* isolates showed a higher percentage of strains resistant to gentamicin (48.1%), vancomycin (55.6%), linezolid (100%), and streptomycin (33.3%) than *E. faecium* isolates. The resistance genes of *Enterococcus* spp. were present to highly varying extents according to antibiotic type, their presence breaking down for *E. faecalis* and *E. faecium* respectively as *aac(6')/aph(2")* 5.56% and 5.56%; *aph(3')-Ⅲ* 0% and 14.81%; *ant(6)-I* 0% and 3.7%; *ant(4')-Ia* 0% and 64.81%; *tetL* 20.37% and 100%; *vanA* 92.59% and 46.3%; *vanB* 0% and 0%; *cfr* 0% and 90.74%; *optrA* 96.3% and 3.7%; *blaZ* 0% and 1.85%; *blaTEM* 0% and 0%; *tetA* 20.37% and 0%; *tetC* 24.07% and 100%; *tetM* 0% and 0%; *ermA* 12.96% and 100%; *ermB* 5.56% and 3.7%; and *ermC* 0% and 1.85%.Virulence-associated determinants were detected in this research, which typically include *efaA*, *gelE*, *asa1*, *ace*, *cylA*, *esp* and *hyl*; however, the latter three were not detected. High proportions of the isolates carried the *efaA*, *gelE*, *asa1*, and *ace* genes. Respectively for *E. faecalis* and *E. faecium* their detection was *efaA* 98.1% and 85.2%; *gelE* 98.1% and 87%; *asa1* 92.6% and 87%; and *ace* 87% and 85.2%. **Conclusion:** This is the first study on the potential disease risk and antimicrobial-resistant characteristics of *E. faecalis* and *E. faecium* isolates in giant panda oral cavities. The results of this study show that the antimicrobial resistance rate of *Enterococcus* spp. isolated from the oral cavity of captive pandas is very high, and thus needs to be monitored.

**Keywords:** *Enterococcus* spp., giant panda, antimicrobial resistance, resistance genes, virulence factors.

## Introduction

*Enterococcus* spp. are natural bacteria in the gut of both humans and animals. As an opportunistic pathogen, it can cause infection when animal immunity is low. The *Enterococcus* genus presently contains over 50 species, among which *E. faecalis* and *E. faecium* dominate, accounting for more than 80% of isolates. In addition, *Enterococcus* spp. have become the second most common iatrogenic infection causing bacteria after *Staphylococcus aureus*. *E. faecalis* is of great importance as a leading opportunistic pathogen causing nosocomial infections, the frequent types of which include endocarditis, meningitis, and urinary tract, wound, and neonatal infections (2).

While *Enterococcus* spp. are not regarded as normal inhabitants of the oral cavity, they have been isolated from samples from patients with various oral conditions including carious lesions, periodontitis, root canal infection (38) and peri-implantitis (15). Some researchers believe that the pathogenic mechanism of *Enterococcus* spp. in the oral cavity may be related to the ability to form recalcitrant biofilms in the root canal (28) and carry virulence factors. The most studied virulence-associated determinants are aggregation substances, surface adhesins, sex pheromones, lipoteichoic acid,

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production of extracellular superoxide, gelatinase, hyaluronidase, and the cytolysin toxin (21). In addition, *E. faecalis* from the oral cavity not only causes pulp disease, but also has the ability to colonise other tissue and infect it systemically, *e.g.* in the form of endocarditis (25).

Due to their ubiquity in human and animal faeces and persistence in the environment, *Enterococcus* spp. are considered indicators of faecal contamination in water. Moreover, *Enterococcus* spp. serve as important key indicator bacteria for human and veterinary resistance surveillance systems. Antimicrobial-resistant *Enterococcus* spp. have the potential to cause zoonotic diseases, being possessed of intrinsic resistance to various antimicrobial agents including aminoglycosides and cephalosporins, and able to acquire resistance genes from other bacteria by conjugation *via* plasmids or transposons and bacteriophages (11). This phenomenon has led to an increase in the prevalence rate of multidrug resistant (MDR) *Enterococcus* spp.

Information on the antimicrobial susceptibility characteristics of *Enterococcus* spp. isolates from the giant panda oral cavity is scarce. Only a small amount of metagenome analysis has been done on the bacterial composition of this microbiome. The first aim of the present study was to take this analysis further, focusing on selected resistance genes as well as additional relevant phenotypic resistance to assess whether the isolated strains could represent a reservoir for antimicrobial resistance traits. The second aim was to evaluate the major virulence traits of *Enterococcus* spp. isolates.

## Material and Methods

**Bacterial strains.** A total of 108 strains comprising 54 of *E. faecalis* and 54 of *E. faecium* were used for the study and were isolated from sublingual saliva samples of 15 giant pandas. They were collected from captive giant pandas living in the Chengdu Research Base of Giant Panda Breeding in the Sichuan Province, China. All isolates were presumptively identified by phenotypic methods, including Gram staining and *Enterococcus* spp. chromogenic medium (Hopebiol Biotech, Qingdao, China) growth. We used 16 S rDNA sequences for final identification and the confirmed isolates were stored in Luria Bertani broth containing 50% glycerol at −20°C for further analyses.

**Antimicrobial susceptibility test.** Susceptibility to 10 antimicrobial agents (penicillin (10 U), ampicillin (10 μg), ciprofloxacin (5 μg), levofloxacin (5μg), erythromycin (15 μg), gentamicin (120 μg), streptomycin (300 μg), tetracycline (30 μg), linezolid (30 μg), and vancomycin (30 μg)) was assessed using the disk diffusion method according to the criteria of the Clinical and Laboratory Standards Institute (6). Drug-sensitive paper was purchased from Hangzhou Microbial Reagent Co. (Hangzhou, China) and Thermo Fisher Scientific (Waltham, MA, USA). *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 strains were used for quality control. Isolates resistant to at least one member of three different antimicrobial groups were considered MDR (9).

**DNA extraction and screening for antibiotic resistance genes.** Total genomic DNA was extracted from isolates using the TIANamp Bacteria DNA kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. DNA samples were stored at −20°C.

Seventeen antimicrobial resistance genes were detected using PCR. The primers used in this study are shown in Table 1. All the design sequences utilised in this research were found through GenBank, and then Oligo7 was used to design the primers. To amplify the *aac(6')/aph(2")*, *aph(3')-Ⅲ*, *ant(6)-I*, *ant(4')-Ia*, *tetL*, *vanA*, *vanB*, *blaTEM*, *cfr*, *optrA* and *blaZ* genes a single PCR was used. The PCR program and amplification system in part exploit prior knowledge in the literature referenced in Table 1. For detecting the presence of the *tetA*, *tetC*, *tetM*, *ermA*, *ermB*, *and ermC* genes, a multiplex PCR was used according to protocols described previously (3).

The PCR products were separated by gel electrophoresis in a 1.0% agarose gel stained with GoldView (Sangon Biotech, Shanghai, China), visualised under ultraviolet light, and photographed using a gel documentation system (Bio-Rad, Hercules, CA, USA).

**Detection of virulence-associated determinants.** Bacterial DNA extract was thawed immediately before performing PCR. Genes encoding the *asa1*, *gelE*, *cylA*, *esp*, *hyl*, *ace*, and *efaA* enterococcal virulence factors were detected using PCR under conditions described previously (20, 26, 35). All primers are shown in Table 2.

## Results

**Antimicrobial susceptibility.** The results of the resistance of *Enterococcus* strains to selected antimicrobials are given in Fig.1. The 108 isolates from the saliva of giant pandas from Chengdu showed different degrees of resistance to 10 antimicrobials.

The drug resistance rates of the 54 *Enterococcus faecalis* strains were 61.1% to penicillin, 48.1% to gentamicin, 90.7% to erythromycin, 55.6% to vancomycin, 100% to linezolid, 98.1% to ciprofloxacin, 33.3% to streptomycin, 55.6% to ampicillin, 83.3% to tetracycline, and 94.4% to levofloxacin. For the 54 *E. faecium* isolates, the resistance rates of 90.7% to penicillin, 100.0% to erythromycin, 88.9% to ampicillin, 98.1% to tetracycline, and 98.1% to levofloxacin were higher than those of the *E. faecalis* isolates.

**Table 1.** Primers used for PCR detection of antimicrobial resistance genes

| Resistance to | Resistance gene | Primer sequence (5'→3') | Amplicon (bp) | References |
|---|---|---|---|---|
| Aminoglycoside | *aac(6')/aph(2")* | CCAAGAGCAATAAGGGCATA<br>CACTATCATAACCACTACCG | 220 | this study |
| | *aph(3')-III* | GCCGATGTGGATTGCGAAAA<br>GCTTGATCCCCAGTAAGTCA | 292 | this study |
| | *ant(6)-I* | ACTGGCTTAATCAATTTGGG<br>GCCTTTCCGCCACCTCACCG | 597 | (21) |
| | *ant(4')-Ia* | CTTGGACGCTGAGATATATGAGCACC<br>GGAAAGTTGACCAGACATTACGAACT | 294 | (10) |
| Tetracycline | *tetM* | GAGGTCCGTCTGAACTTTGCG<br>AGAAAGGATTTGGCGGCACT | 900 | (21) |
| | *tetA* | GGCACCGAATGCGTATGAT<br>AAGCGAGCGGGTTGAGAG | 480 | (21) |
| | *tetC* | CTGGGCTGCTTCCTAATGC<br>AGCTGTCCCTGATGGTCGT | 580 | (21) |
| | *tetL* | TGGTCCTATCTTCTACTCATTC<br>TTCCGATTTCGGCAGTAC | 385 | (14) |
| Vancomycin | *vanA* | GGGAAAACGACAATTGC<br>GTACAATGCGGCCGTTA | 732 | (14) |
| | *vanB* | CAAAGCTCCGCAGCTTGCATG<br>TGCATCCAAGCACCCGATATAC | 484 | (14) |
| *β*-lactams | *blaZ* | ACTTCAACACCTGCTGCTTTC<br>TAGGTTCAGATTGGCCCTTAG | 240 | (34) |
| | *blaTEM* | CCAATGCTTAATCAGTGAGG<br>ATGAGTATTCAACATTTCCG | 858 | (23) |
| Erythromycin | *ermA* | TCTAAAAAGCATGTAAAAGAAA<br>CGATACTTTTTGTAGTCCTTC | 553 | this study |
| | *ermB* | CCGTTTACGAAATTGGAACAGGTAAAGGGC<br>GAATCGAGACTTGAGTGTGC | 359 | this study |
| | *ermC* | GCTAATATTGTTTAAATCGTCAATTCC<br>GGATCAGGAAAAGGACATTTTAC | 460 | this study |
| Linezolid | *cfr* | TGAAGTATAAAGCAGGTTGGGAGTCA<br>ACCATATAATTGACCACAAGCAGC | 746 | (32) |
| | *optrA* | AGGTGGTCAGCGAACTAA<br>ATCAACTGTTCCCATTCA | 1395 | (5) |

**Table 2.** Primers for different virulence genes

| Virulence factor | Genes | Primer sequence (5′→3′) | PCR product size (bp) | References |
|---|---|---|---|---|
| Aggregation substance | *asa1* | GCACGCTATTACGAACTATGA<br>TAAGAAAGAACATCACCACGA | 375 | (4) |
| Gelatinase | *gelE* | TATGACAATGCTTTTTGGGAT<br>AGATGCACCCGAAATAATATA | 213 | (4) |
| Cytolysin | *cylA* | ACTCGGGGATTGATAGGC<br>GCTGCTAAAGCTGCGCTT | 688 | (4) |
| Enterococcal surface protein | *esp* | AGATTTCATCTTTGATTCTTGG<br>AATTGATTCTTTAGCATCTGG | 510 | (4 |
| Hyaluronidase | *hyl* | ACAGAAGAGCTGCAGGAAATG<br>GACTGACGTCCAAGTTTCCAA | 276 | (4) |
| Accessory colonization factor | *ace* | GAATTGAGCAAAAGTTCAATCG<br>GTCTGTCTTTTCACTTGTTTC | 1108 | (21) |
| Endocarditis antigen | *efaA* | GCCAATTGGGACAGACCCTC<br>CGCCTTCTGTTCCTTCTTTGGC | 688 | (21) |

It is worth noting that all 108 *Enterococcus* spp. isolates were resistant to three or more classes of antimicrobials. Among the *E. faecalis* strains, 34 (62.96%) isolates were resistant to six different antimicrobial agents, whereas among *E. faecium* strains only 25 (46.3%) isolates were found to be resistant to the same number of antimicrobials.

**Antibiotic resistance genes.** The results of investigation of the presence of resistance genes are summarised in Fig. 2. The detection rates of the *tetL* and *tetK* genes in *E. faecalis* isolates were 20.37% and 24.07%, respectively. However, *tetL* and *tetC* were detected in all *E. faecium* isolates. The *tetM* gene was not present in any strain of either bacterium.

The *ermA* gene was detected in all 54 *E. faecium* isolates and in 12.96% of those of *E. faecalis*. In contrast, the detection rates of the *ermB* and *ermC* genes in the *E. faecium* isolates were very low at 3.7% and

1.85%, respectively. In *E. faecalis* isolates, the detection rate of the *ermB* gene was 5.56%, while *ermC* was not detected. The *β*-lactam resistance gene was in very low presence, with a detection rate of only 1.85% in *E. faecium* isolates.

Among *E. faecalis* isolates, the *vanA* gene was found in 50 strains (92.59%), whereas the *vanB* gene was not detected. In *E. faecium* isolates, the detection rate of the *vanA* gene decreased to 46.3%, while the *vanB* gene was likewise not detected. For aminoglycoside drugs, we selected four common resistance genes; namely, *ant6-I*, *ant3'-III*, *aac6'-aph2''*, and *ant(4')-Ia*. Among the *E. faecalis* isolates, only the *aac6'-aph2''* gene was found, and it was identified in 3 strains (5.56%). The detection rates of the aminoglycosides resistance genes in *E. faecium* were 3.7% for *ant6-I*, 14.81% for *ant3'-I*, 5.56% for *aac6'-aph2'',* and 64.81% for *ant(4')-Ia*.

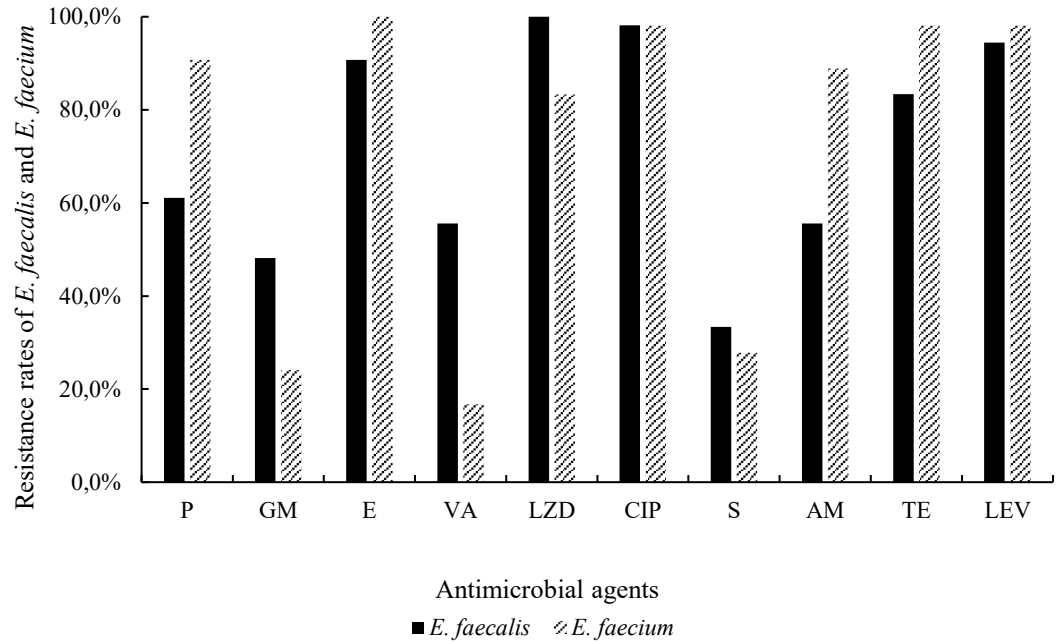


**Fig. 1.** Resistance rates of 54 *E. faecalis* and 54 *E. faecium* strains to 10 antibiotics
P – penicillin; GM120 – gentamicin (120μg); E – erythromycin; VA – vancomycin; LZD – linezolid; CIP – ciprofloxacin; S300 – streptomycin (300μg); AM – ampicillin; TE – tetracycline; LEV – levofloxacin

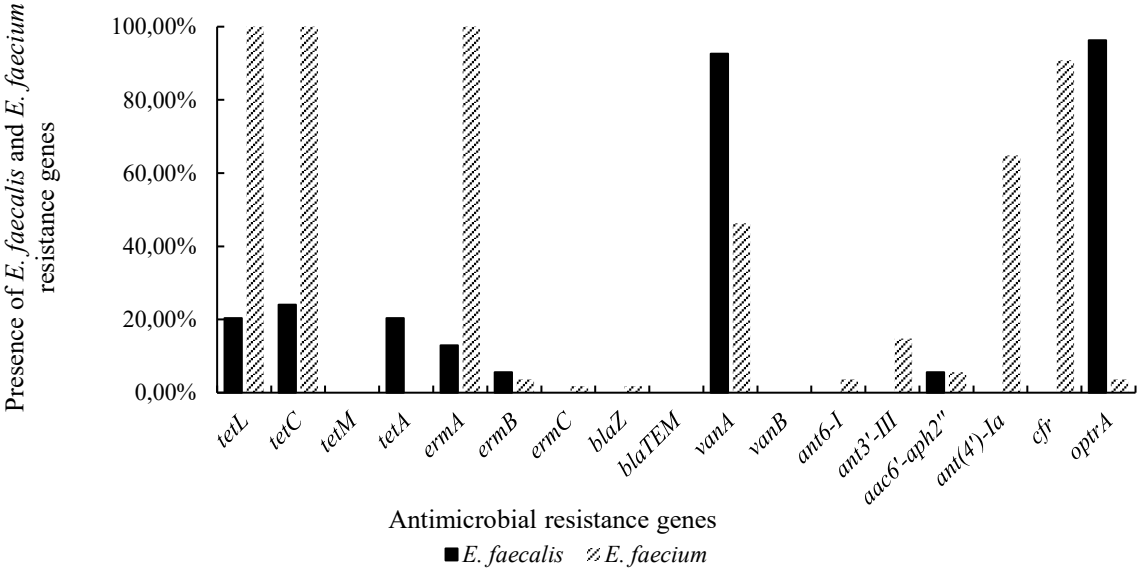


**Fig. 3.** Presence of *E. faecalis* and *E. faecium* resistance genes
*tetL, tetC, tetM,* and *tetA* – tetracycline resistance genes; *ermA, ermB*, and *ermC* – erythromycin resistance genes; *blaZ* and *bla*TEM – *β*-lactam resistance genes; *vanA* and *vanB* – vancomycin resistance genes; *ant6- I , ant3'- III, aac6'-aph2''*, and *ant(4')-Ia* – aminoglycoside resistance genes; *cfr* and *optrA* – linezolid resistance genes

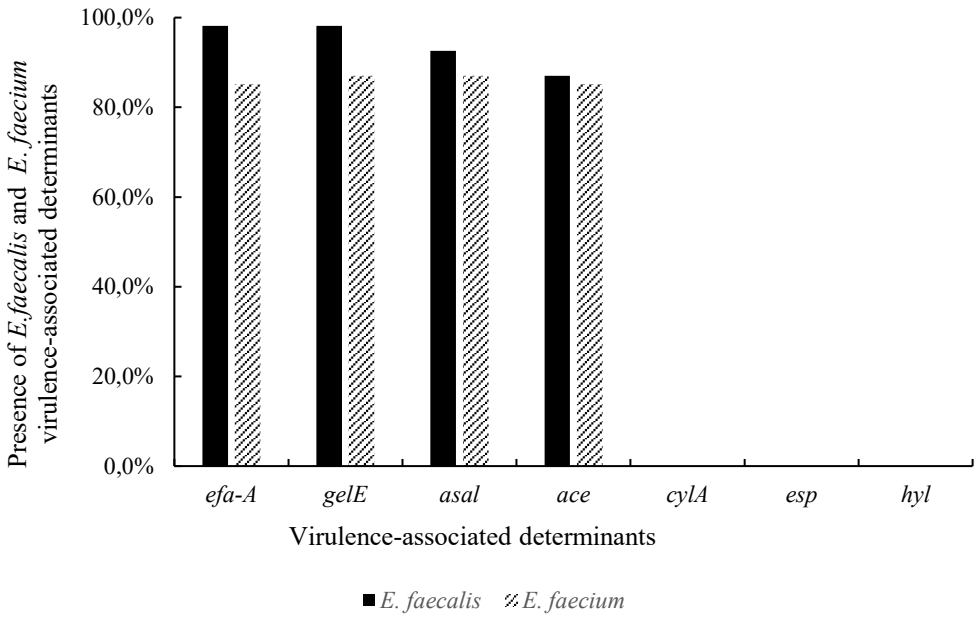


**Fig. 3.** Presence of *E. faecalis* and *E. faecium* virulence-associated determinants
*ace* – collagen-binding protein; *asa1* – aggregation substance; *cylA* – cytolysin; *efa-A* – endocarditis antigen; *esp* – enterococcal surface protein; *gelE* – gelatinase; *hyl* – hyaluronidase

Finally, it is noteworthy that the drug resistance rates of isolates to linezolid were very high in antimicrobial susceptibility test. Both the genes conferring resistance, *cfr* and *optrA*, were detected in PCR. However, the detection rate of *cfr* gene in *E. faecium* isolates was as high as 90.74%, but no *cfr* gene was detected in those of *E. faecalis*. The opposite was true for the *optrA* gene, which was detected at a rate of only 3.7% in *E. faecium* isolates, but at a very high rate of 96.30% in those of *E. faecalis*.

**Virulence-associated determinants.** The results of investigation of the presence of virulence-associated determinants are summarised in Fig. 3.

We tested for the presence of seven virulence factors. The *cylA*, *esp a*nd *hyl* genes were not detected. All 54 *E. faecalis* strains yielded the *efaA*, *gelE*, *asal*, and *ace* genes in abundance, with respective 98.1%, 98.1%, 92.6% and 87.0% detection rates. The same phenomenon was also observed in the 54 *E. faecium* strains, but the detection rates for these four genes were lower. The most common type of virulence factor carrier was *efaA-gelE-asal-ace* among both *E. faecalis* and *E. faecium* (Tables 3 and 4).

**Table 3.** Virulence-associated gene profile of *E. faecalis* isolates from giant panda saliva samples

| Virulence-associated gene | Number of isolates | Proportion |
|---|---|---|
| *efaA-gelE* | 2 | 3.70% |
| *gelE-asal* | 1 | 1.85% |
| *efaA-ace* | 1 | 1.85% |
| *efaA-gelE-ace* | 1 | 1.85% |
| *efaA-gelE-asa1* | 4 | 7.41% |
| *efaA-gelE-asal-ace* | 45 | 83.33% |

**Table 4.** Virulence-associated gene profile of *E. faecium* isolates from giant panda saliva samples

| Virulence-associated gene | Number of isolates | Proportion |
|---|---|---|
| *efaA-ace* | 1 | 1.85% |
| *gelE-asal* | 2 | 3.70% |
| *efaA-gelE-asal-ace* | 45 | 83.33% |
| None | 6 | 11.11% |

## Discussion

In view of the universal finding of MDR in all the isolates tested and the 34 out of 54 (62.96%) *E. faecalis* and 25 out of 54 (46.30%) *E. faecium* strains found to be resistant to six to seven antibiotics, high rates of drug resistance exist in *Enterococcus* spp. colonising captive giant panda oral cavities, and indicate a severe problem.

Since the 1990s, *Enterococcus* spp. have emerged as leading nosocomial pathogens and been shown to have the ability to acquire and spread resistance genes readily (38). However, the role of *Enterococcus* spp. such as *E. faecalis* that inhabit the oral cavity as a potential reservoir for resistance has not been clarified yet. The emergence of *Enterococcus* isolates that have multidrug resistance phenotypes, which confer resistance to three or more unrelated families of antibiotics, is considered a serious problem. Increasing resistance to antimicrobials of which tetracycline, rifampicin, ciprofloxacin, and erythromycin are some examples has been reported in *E. faecalis* (1). The results of this study showed that 98% of the *E. faecalis* strains and 98% of the *E. faecium* strains were resistant to ciprofloxacin, which is much higher than the 25% resistance rate reported in India (31) and 38.1% in Portugal (18). Previous studies have suggested that the

rampant use of fluoroquinolones has contributed to the emergence of high-level or complete resistance and a high prevalence of MDR (13). Such observations have also been reported in previous studies of human cases of enterococcal urinary tract infections (16). The resistance rate to levofloxacin at 94.4% was like the rate to ciprofloxacin. Erythromycin, tetracycline and linezolid were found to be resisted by the bacteria in this study as much as quinolones. In our study, 90.7% of *E. faecalis* isolates and 100% of *E. faecium* isolates were resistant to erythromycin. This result is similar to that of Sattari-Maraji *et al.* (30), for whom the resistance rate to erythromycin was close to 100%. The *ermA* gene had the highest detection rates of the erythromycin resistance genes, being present in 12.96% and 100% respectively of *E. faecalis* and *E. faecium* isolates. Only low inclusions of *ermB* and *ermC* were detected, which was inconsistent with the high detection rate of *ermB* reported by Guerrero-Ramos *et al.* (10) and Bin *et al.* (14) in meat products.

The resistance rates of *E. faecalis* and *E. faecium* isolates to tetracycline were as high as 83.3% and 98.1%. The common tetracycline resistance genes *tetL*, *tetC*, *tetA* and *tetM* were selected for detection. The rate at which *tetL* and *tetC* were detected was high, but the *tetM* carriage rate was 0%. This is inconsistent with the high detection rates of these genes in isolated strains observed in hospitals by Tian *et al.* (33).

The *optrA* gene, which confers transferable resistance to oxazolidinones (linezolid and tedizolid) and phenicols (chloramphenicol and florfenicol), has been detected in *E. faecalis* and *E. faecium* isolates of both human and animal origin. This gene encodes an ABC transporter and has been detected more frequently in *E. faecalis* than in *E. faecium* isolates. The *cfr* gene also confers the same resistance; it encodes an rRNA methyltransferase that modifies the adenine residue at position 2503 in domain V of the 23S rRNA. Besides resistance to oxazolidinones and phenicols, it also confers resistance to lincosamides, pleuromutilins, and streptogramin A (23). The spread of these genes could significantly limit treatment options for MDR bacteria infections (33). In our experiments, the resistance of *E. faecalis* and *E. faecium* to linezolid was also very high, reaching 100% and 83%. The detection rates of *optrA* and *cfr* were extreme opposites in the two enterococcal species, with detection rates of 0% and 96.3% in *E. faecalis* and 90.74% and 3.7% in *E. faecium*. The detection of these genes in *E. faecalis* was similar to that of Chen *et al.* (5) in a linezolid-resistant strain. However, in most reports, *Enterococcus* spp. are still susceptible to linezolid (4, 9, 37).

Enterococci have different resistance strengths to different types of *β*-lactam antibiotics (16). In our study, antimicrobial susceptibility tests to penicillin and ampicillin were carried out, and demonstrated prevalence rates of *E. faecalis* and *E. faecium* resistant to ampicillin of 55.6% and 88.9%, respectively and rates for the isolates resistant to penicillin of 61.1% and 90.7%. *Enterococcus faecalis* isolates were more susceptible to *β*-lactams than *E. faecium* isolates, but susceptibility to aminoglycosides was higher in *E. faecium* isolates than *E. faecalis* isolates. The drug resistance rates of *E. faecalis* to gentamicin and streptomycin were 48.2% and 33.3% and those of *E. faecium* were 24.1% and 27.8%.

For vancomycin resistance, we observed significant differences in the susceptibility of *E. faecalis* and *E. faecium*, with resistance rates of 55.6% and 16.6%, respectively. The two genotypes *vanA* and *vanB* are the most frequent among vancomycin-resistant strains. The detection rates of *E. faecalis* and *E. faecium* with the *vanA* gene were 92.59% and 46.3%, respectively, while isolates with *vanB* were not detected, which was inconsistent with the resistance rate to vancomycins. Ribeiro *et al.* (29) reported that even when the *vanA* gene was detected, there was no resistance to vancomycin. In an investigation of oral dental diseases, oral isolates of *E. faecalis* were sensitive to vancomycin, which was a favourable finding (27). At the same time, we observed that the isolates were resistant to antibiotics that were no longer used; the possible explanation might be the incorporation of resistance genes into the host chromosome or the physical linkage of the antibiotic genes on the plasmid.

The presence of virulence-associated determinants and antibiotic-resistant phenotypes may enhance the pathogenesis of the *Enterococcus* strains due to increased adhesion, colonisation, extracellular production of enzymes, and evasion of the host immune response.

*E. faecalis* isolates harboured significantly more virulence-associated determinants than *E. faecium* isolates in previously reported data (36). In our experiment, only four virulence-associated determinants were detected; namely, the *efaA*, *gelE*, *asa1*, and *ace* genes, but the detection rate for them was above 85% and higher in *E. faecalis* than in *E. faecium*. The virulence-associated determinants carried by 83.33% (90/108) of the isolates were of *efaA-gelE-asa1-ace* type. In our study, *gelE* was extensively present in both *E. faecalis* and *E. faecium* isolates (98.1% and 87.0%, respectively), similarly to the results of Landete *et al.* (17) (81% and 60%, respectively).

Genes for enterococcal surface protein and cell wall adhesins (*espfm*, *espfs*, *efaAfm*, and *efaAfs*) were as frequent in their corresponding species as they were found to be by Togay *et al.* (34). In our study, *efaA* was found in 98.1% of *E. faecalis* and 85.2% of *E. faecium* strains, but the *esp* gene was not detected. This phenomenon is a contrary finding to that of Creti *et al.* (7) and Martin *et al.* (19), who both discovered a high incidence of these genes in *Enterococcus* spp. isolates. In this study, up to 98.1% of *E. faecalis* isolates were *gelE*-positive and 92.6% were *asa1*-positive, which is consistent with previous studies and provides further evidence that these virulence-associated determinants are widely distributed among *E. faecalis* strains (24).

Interestingly, the *hyl* and *cylA* gene detection rates were 0%, making results similar to those of Creti *et al.* (7) but consistent with other previous studies (22). An 87% proportion of *E. faecalis* isolates and 85.2% of *E. faecium* isolates were *ace*-positive.

In summary, our data illustrate that giant panda saliva presents a reservoir of *Enterococcus* spp. strains with multi-drug resistance and these isolates carry some virulence-associated determinants that may increase the risk of disease. Consequently, continued monitoring of *Enterococcus* spp. for antibiotic resistance and virulence-associated determinants should be performed in giant pandas' oral cavities that will help to establish strategies for prevention and surveillance of greater virulence and resistance in these bacteria as pathogens for this endangered species.

**Conflict of Interests Statement:** The authors declare that there is no conflict of interests regarding the publication of this article.

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**Animal Rights Statement:** This study protocol was reviewed and approved by the Research Ethics Committee and the Animal Ethical Committee of Sichuan Agricultural University under permit number DYY-2018403006. Prior to the collection of saliva specimens from captive giant pandas, permission was obtained from the Chengdu Research Base of Giant Panda Breeding in the Sichuan Province, China.

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