

# Prevalence and Molecular Characteristics of Enterococci Isolated from Clinical Bovine Mastitis Cases in Ningxia

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**Purpose:** This study aimed to investigate the prevalence and genetic characterization of enterococcal isolates (*Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus hirae*) isolated from clinical bovine mastitis cases in Ningxia, China.

**Patients and Methods:** The enterococci were identified by 16S rRNA amplification and sequencing. Antimicrobial resistance was determined by disc diffusion method. Virulence and antimicrobial resistance genes were detected by PCR assays.

**Results:** Overall, 198 enterococcal isolates were identified from 2897 mastitis samples, including 137 (4.7%) *E. faecalis*, 50 (1.7%) *E. faecium* and 11 (0.4%) *E. hirae*. *E. faecalis*, *E. faecium* and *E. hirae* isolates showed high resistance to tetracycline (92.7%, 68.0%, 90.9%), followed by erythromycin (86.9%, 76.0%, 72.7%). The multidrug-resistant strains of *E. faecalis* and *E. faecium* were 29 (21.2%) and 13 (26.0%), respectively. The resistance of *E. faecalis*, *E. faecium* and *E. hirae* isolates to tetracycline is mainly attributed to the presence of *tetL* (alone or combined with *tetM* and/or *tetK*), the erythromycin resistance to *ermB* (alone or combined with *ermC* and/or *ermA*). Moreover, *cpd* (94.2%), *gelE* (77.4%), *efaAfs* (93.4%), and *esp* (79.6%) were the most common virulence genes in *E. faecalis*. In *E. faecium*, except for the gene *efaAfs* (82.0%), other virulence genes are rarely found. Only two strains of *E. hirae* carrying *asaI* gene were detected.

**Conclusion:** The results of this study can provide a reference for the prevention and treatment of bovine mastitis caused by enterococci.

**Keywords:** bovine mastitis, enterococci, antimicrobial resistance, virulence

## Introduction

Mastitis is the most common disease of bovines, and it adversely affects animal health and the profitability of dairy farms, including increases in bovine mortality and decreases in milk production.<sup>1</sup> Greater progress has been made in controlling infectious mastitis pathogens through improved milking room hygiene and feeding management, but for opportunistic mastitis pathogens present in the environment, such as enterococci, these management measures are less effective. Enterococci is a facultative anaerobic gram-positive bacteria found in intestines of humans and animals. According to the consistency of 16S rRNA sequence, enterococci can be divided into several subgroups, such as *E. faecalis*, *E. faecium*, *E. hirae*. They are highly tolerant to the environment and have a variety of host niches. Several studies in the past have shown that enterococci can cause mastitis in dairy bovines.<sup>2-4</sup>

At present, antibiotics are still the main treatment for bovine mastitis. However, this method can lead to an increased incidence of antimicrobial resistant infections in bovines during long-term rearing, increasing production costs and even causing public health incidents. Enterococci could develop resistance to most existing antibiotics by mutation or incorporation of foreign genetic material,<sup>5</sup> and it may be possible for these antibiotic resistances to be transmitted in other bacteria by exchange of genetic information (plasmids and transposon) through mobile genetic elements.<sup>6</sup> Previous studies have reported that *E. faecium* isolated from food is resistant to a variety of antibiotics, including aminoglycosides, macrolide,  $\beta$ -lactam, and tetracycline.<sup>7</sup> Recent studies have attributed enterococci toxicity to various factors, such as gelatinases, surface proteins, sex pheromones, collagen-binding cell wall proteins, polymeric substances, and biofilm formation.<sup>2</sup>

At present, enterococci have been reported worldwide. Due to the diversity and variability of enterococci genes,<sup>8</sup> a deeper understanding of the genotype of enterococci and its epidemiology in dairy bovines may help to monitor the emergence of highly virulent strains or antibiotic resistant strains. The purpose of this study was to study the prevalence, antimicrobial resistance, and virulence characteristics of predominant enterococci (*E. faecalis*, *E. faecium* and *E. hirae*) isolated from clinical bovine mastitis in Ningxia Hui Autonomous Region of China.

## Materials and Methods

### Sample Collection and Enterococci Isolates

Mastitis milk samples of dairy cows (n=2897) were collected from 21 commercial dairy herds in Ningxia Hui Autonomous Region, China, from July 2018 to May 2023. Clinical mastitis cases were confirmed by the abnormal signs of bovine udder (oedema, lumps, increase in temperature, hardening or pain) and milk samples (presence of clots, flakes or blood).<sup>9</sup> After transportation to the laboratory, 100  $\mu$ L of each sample was plated on sheep blood agar (Huan kai, Guangdong, China) at 37 °C aerobically for 18 to 24 h. Colonies were initially identified as enterococcal isolates based on the morphological features (shape, color, and size).<sup>10–13</sup> The assumed isolates were further confirmed by PCR and sequencing as described in our previous study.<sup>9</sup> Briefly, the genomic DNA was extracted by the Bacterial DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The 16S rRNA gene was amplified by the 16S rDNA Bacterial Identification PCR Kit (Takara, Shiga, Japan) according the manufacturer's recommendation. The PCR products were purified and sequenced through Sanger sequencing (Tsingke, Xi'an, China). BLAST analysis tool from NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to analyze the nucleotide sequences obtained.

### Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of enterococcal isolates to 8 antimicrobial agents was determined using the disk diffusion method on Mueller Hinton agar (Oxoid, Basingstoke, UK) plates according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2018). The (Oxoid) antibiotic discs included tetracycline (30  $\mu$ g), vancomycin (30  $\mu$ g), penicillin (10  $\mu$ g), quinupristin (15  $\mu$ g), erythromycin (15  $\mu$ g), ciprofloxacin (5  $\mu$ g), chloramphenicol (30  $\mu$ g), and rifampin (5  $\mu$ g). Multidrug-resistant (MDR) isolates were defined as an isolate being resistant to at least 3 antimicrobial agents belonging to different antimicrobial categories (Magiorakos et al, 2012). *Staphylococcus aureus* ATCC 25923 was used as quality control strains.

### Genetic Determinants for Antimicrobial Resistance and Virulence

Resistance genes and virulence genes were detected by PCR, and primers and thermal conditions were summarized in [Table S1](#). The resistance-associated genes for tetracycline (*tetK*, *tetL*, *tetM*, *tetO* and *tetS*) and erythromycin (*ermA*, *ermB*, and *ermC*) were searched using PCR as previously described.<sup>14–19</sup> The virulence factors sex pheromone determinant (*cpd*), cytolysin (*cylA*), gelatinase (*gelE*), regulator of the expression of *gelE* (*frs*), hyaluronidase (*hyl*), cell wall adhesins (*efaAfs*, *efaAfm*, *efaA*), aggregation substance (*asaI*), enterococcal surface protein (*esp*), collagen adhesion (*ace*) were also evaluated through PCR.<sup>20–22</sup> PCR products (10  $\mu$ L) were analyzed by electrophoresis on 1.2% agarose gel and photographed using a UV imaging system.

## Results

### Antimicrobial Susceptibility of Enterococci

In this study, a total of 198 enterococcal isolates (6.8%) were isolated from 2897 clinical mastitic samples, including *E. faecalis* (137, 4.7%), *E. faecium* 50 (1.7%) and *E. hirae* 11 (0.4%). The antimicrobial susceptibility of these isolates to 8 antibiotics was evaluated and summarized in Table 1. The results showed that 171 (86.4%) enterococcal isolates were resistant to tetracycline (92.7% for *E. faecalis*; 68.0% for *E. faecium*; 90.9% for *E. hirae*) and 166 (83.8%) to erythromycin (86.9% for *E. faecalis*; 76.0% for *E. faecium*; 72.7% for *E. hirae*). Resistance against rifampin and chloramphenicol was found in 45 (22.7%) and 23 (11.6%) isolates, respectively. Quinupristin-dalfopristin, ciprofloxacin and penicillin resistance were measured in 30 (15.2%), 24 (12.1%) and 10 (5.1%) of the enterococcal isolates except for *E. hirae*, respectively. None of the isolates showed resistance to vancomycin. In addition, multidrug resistance was found in 42 (21.2%) enterococcal isolates, including *E. faecalis* (29, 21.2%) and *E. faecium* (13, 26.0%).

### Genotypic Resistance Profiles of Enterococci

In this study, the enterococcal isolates showed higher resistance to tetracycline and erythromycin in comparison with other tested antimicrobial agents. Therefore, we tested the antimicrobial resistance gene frequencies of tetracycline (*tetK*, *tetL*, *tetM*, *tetO*, and *tetS*) and erythromycin (*ermA*, *ermB*, and *ermC*) (Table 2). The tetracycline-resistant gene *tetL* was detected in 196 (99.0%) enterococcal isolates, followed by *tetM* (170, 85.9%), *tetK* (118, 59.6%), *tetS* (90, 45.5%) and *tetO* (18, 9.1%). One hundred and ninety-six *tetL* alone or combined with *tetM*, *tetK*, *tetS* and/or *tetO* was found in all tetracycline-resistant isolates. However, 25 *tetL*-carrying (alone or combined with *tetM*, *tetK*, *tetL*, *tetS* and/or *tetO*) isolates, including 10 *E. faecalis*, 14 *E. faecium*, and 1 *E. hirae*, were susceptible to tetracycline. Additionally, *ermB*, *ermC* and *ermA* were found in 189 (95.5%), 14 (7.1%) and 2 (1.0%) enterococcal isolates, respectively. One hundred and ninety *ermB* alone or combined with *ermC* and/or *ermA* was found in all erythromycin-resistant isolates. However, 23 *ermB*-carrying (alone or combined with *ermC* and/or *ermA*) isolates, including 10 *E. faecalis*, 11 *E. faecium*, and 3 *E. hirae*, were susceptible to erythromycin.

### Genotypic Virulence Profiles of Enterococci

The presence of virulent genes in enterococcal isolates are shown in Table 3. *E. faecalis* isolates carried most of the tested genes, including *cpd* (129, 94.2%), *efaAfs* (128, 93.4%), *esp* (109, 79.6%), *gelE* (106, 77.4%), *ace* (43, 31.6%), *efaAfm*

**Table 1** Antimicrobial Resistance Characteristics of Enterococci Isolates

Species (No.)	TET	VAN	PEN	QDA	ERY	CIP	CHL	RIF	MDR
<i>Enterococcus faecalis</i> (137)	127 (92.7%)	0 (0)	1 (0.7%)	27 (19.7%)	119 (86.9%)	8 (5.8%)	18 (13.1%)	29 (21.2%)	29 (21.2%)
<i>Enterococcus faecium</i> (50)	34 (68.0%)	0 (0)	9 (18.00%)	3 (6.0%)	39 (76.0%)	16 (32.0%)	4 (8.0%)	14 (28.0%)	13 (26.0%)
<i>Enterococcus hirae</i> (11)	10 (90.9%)	0 (0)	0 (0)	0 (0)	8 (72.7%)	0 (0)	1 (9.1%)	2 (18.2%)	0 (0)
Total (198)	171 (86.4%)	0 (0)	10 (5.05%)	30 (15.2%)	166 (83.8%)	24 (12.1%)	23 (11.6%)	45 (22.7%)	42 (21.2%)

**Abbreviations:** TET, tetracycline; VAN, vancomycin; PEN, penicillin; QDA, Quinupristin-dalfopristin; ERY, erythromycin; CIP, ciprofloxacin; CHL, chloramphenicol; RIF, rifampin; MDR, Multidrug-resistant.

**Table 2** Characterization of Drug Resistance Genes in Enterococci Isolates

Species (No.)	TET					ERM		
	<i>tetK</i>	<i>tetL</i>	<i>tetM</i>	<i>tetO</i>	<i>tetS</i>	<i>ermA</i>	<i>ermB</i>	<i>ermC</i>
<i>Enterococcus faecalis</i> (137)	90 (65.7%)	137 (100.0%)	125 (91.2%)	4 (2.9%)	61 (44.5%)	1 (0.7%)	130 (94.9%)	4 (2.9%)
<i>Enterococcus faecium</i> (50)	24 (48.0%)	48 (96.0%)	38 (76.0%)	12 (24.0%)	23 (46.0%)	1 (2.0%)	49 (98.0%)	7 (14.0%)
<i>Enterococcus hirae</i> (11)	4 (36.4%)	11 (100.0%)	7 (63.6%)	2 (18.2%)	6 (54.5%)	0 (0)	10 (90.9%)	3 (27.3%)
Total (198)	118 (59.6%)	196 (99.0%)	170 (85.9%)	18 (9.1%)	90 (45.5%)	2 (1.0%)	189 (95.5%)	14 (7.1%)

**Table 3** Characterization of Virulence Genes in Enterococci Isolates

Species (No.)	<i>cpd</i>	<i>cylA</i>	<i>gelE</i>	<i>frs</i>	<i>hyl</i>	<i>efaAfs</i>	<i>efaAfm</i>	<i>efaA</i>	<i>asaI</i>	<i>esp</i>	<i>ace</i>
<i>Enterococcus faecalis</i> (137)	129 (94.2%)	22 (16.1%)	106 (77.4%)	0 (0)	0 (0)	128 (93.4%)	38 (27.7%)	0 (0)	38 (27.7%)	109 (79.6%)	43 (31.4%)
<i>Enterococcus faecium</i> (50)	2 (4.0%)	0 (0)	0 (0)	0 (0)	0 (0)	41 (82.0%)	3 (6.0%)	0 (0)	8 (16.0%)	2 (4.0%)	2 (4.0%)
<i>Enterococcus hirae</i> (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (18.2%)	0 (0)	0 (0)
Total (198)	131 (66.2%)	22 (11.1%)	106 (53.5%)	0 (0)	0 (0)	169 (85.4%)	41 (20.7%)	0 (0)	48 (24.2%)	111 (56.1%)	45 (22.7%)

(38, 27.7%), *asaI* (38, 27.7%) and *cylA* (22, 16.1%). In *E. faecium*, the *efaAfs* (41, 82.0%) was the most prevalent virulence gene, followed by *asaI* (8, 16.0%), *efaAfm* (3, 6.0%), *cpd* (2, 4.0%), *esp* (2, 4.0%), and *ace* (2, 4.0%). However, aside from 2 *asaI*-positive *E. hirae* isolates, none of the other virulent genes were detected in this species.

## Discussion

Enterococci are the main environmental pathogens causing bovine mastitis and antimicrobials are widely used for the treatment of these infections.<sup>23,24</sup> Unfortunately, enterococcus has inherent resistance to many broad-spectrum antibiotics, posing a significant threat to public health.<sup>25</sup> Understanding the prevalence, antimicrobial resistance and virulence factors of enterococci is of great value for targeted therapy and control of bovine mastitis. In the present study, *E. faecalis*, *E. faecium*, and *E. hirae* were detected in 4.7%, 1.7%, and 0.4% of 2897 mastitic samples, respectively. This is a slight increase from the prevalence of *E. faecalis* (4.5%) reported in previous studies in China.<sup>2</sup> It is lower than rates reported in California, USA,<sup>26</sup> but higher than the *E. faecalis* (0.2%), *E. faecium* (0.2%) reported in Brandenburg, Germany.<sup>27</sup> The management system of the farm, source of samples, climate, and herd size may explain some differences in the research results.<sup>28</sup> As reported in the past, the prevalence of *E. hirae* is much lower than that of *E. faecalis* and *E. faecium*.<sup>29,30</sup> It is worth noting that recent research has shown that *E. hirae* is a cause of infection in various animal diseases.<sup>31</sup> At present, reports of *E. hirae* are limited worldwide, resulting in a lack of comprehensive reports on clinical features and management.<sup>32</sup> This study supplements the clinical characteristics of *E. faecalis*, *E. faecium*, and *E. hirae*, which causes mastitis in northwest China.

Antibiotic therapy has been used as one of the successful strategies to control mastitis caused by enterococci.<sup>33</sup> The  $\beta$ -Lactam, tetracyclines, macrolides and aminoglycosides are commonly used to treat bovine mastitis.<sup>34</sup> Unfortunately, the increase in the number of drug-resistant strains hinders the effectiveness of antibacterial treatment.<sup>35</sup> It is worth noting that previous studies have reported that *E. faecalis* is prone to acquire antimicrobial resistance.<sup>15</sup> Our study found that *E. faecalis*, *E. faecium* and *E. hirae* showed high resistance to tetracycline and erythromycin. These data are similar to those contained in other reports.<sup>2,36–38</sup> In addition, previous studies have also reported high resistance to tetracycline and erythromycin in *E. faecalis* isolated from food animals.<sup>15,39</sup> The reason of this result may be due to the long-term and widespread use of tetracycline and erythromycin in non-targeted therapies.<sup>2,40</sup> Thus, antimicrobials such as tetracycline and erythromycin should be used cautiously when treating cow mastitis caused by enterococci in Ningxia, China. In the present survey, the *E. faecium* and *E. hirae* isolates displayed fewer resistance phenotypes than the *E. faecalis*. This is consistent with previous research results.<sup>41</sup> Besides, our results indicate that *E. faecalis* and *E. faecium* have relatively low resistance rates to vancomycin, quinupristin-dalfopristin, ciprofloxacin, chloramphenicol, and rifampicin. The lower proportion of antibiotic resistance may be due to the lower frequency of use of these antibiotics in dairy farms. However, the multidrug resistant strains were still observed in 21.2% and 26.0% of the *E. faecalis* and *E. faecium*, respectively. This result suggests that we should use antibiotics with caution. Because this may be due to increasing exposure to antimicrobial environments can lead to an increase in the number of multidrug-resistant strains. Therefore, the frequent use of these antibiotics in the treatment of mastitis or other diseases may explain the high phenotype of multidrug resistance. Previous studies have shown that enterococci is a common multidrug resistant bacterium.<sup>42,43</sup> Enterococci may transfer resistance genes to more pathogenic bacteria through mobile genetic elements (MGE).<sup>44</sup> Therefore, these resistant agricultural isolates may be important stepping stones in the evolution of enterococci into resistant human pathogens.<sup>45</sup>

In this study, the most commonly antimicrobial resistance of the three enterococcal species identified was resistance to erythromycin and tetracycline. Therefore, this study tested the genes that develop the corresponding resistance. The resistance of enterococci to tetracyclines is mainly mediated by the *tet* genes. In this study, the *tetL* and *tetM* gene was the most prevalent gene among the tetracycline-resistant isolates. The *tetL* and *tetM* gene has been found to be widespread among enterococci and is often associated with high-level tetracycline resistance.<sup>46</sup> In addition, the *tet* genes are usually carried on plasmids or transposons, which can easily spread between bacteria, leading to the dissemination of antibiotic resistance.<sup>47</sup> The resistance of enterococci to macrolides is mainly mediated by the *erm* genes.<sup>48</sup> The *erm* gene modifies the target site on the bacterial ribosome to prevent the binding of the antibiotics, thereby producing a high level of resistance to macrolides.<sup>26</sup> In this study, the *ermB* gene was the most prevalent gene among the erythromycin-resistant isolates. The *ermB* genes encode methylases, which induces N<sup>6</sup>-dimethylation at A2071 of the 23S rRNA,<sup>49</sup> leading to the inhibition of macrolide binding.<sup>50</sup> The *erm* genes are usually carried on conjugative transposons, which can move between bacteria and spread antibiotic resistance.<sup>51</sup> The results of antibiotics resistance gene testing in this study were supported by previous studies, and *tetL*, *tetM*, and *ermB* were more commonly observed in enterococci.<sup>36,52,53</sup> It is noteworthy that 25 isolates carrying at least one *tet* (*tetK*, *tetL*, *tetM*, *tetO*, and *tetS*) gene are sensitive to tetracycline, and 23 isolates carrying at least one *erm* (*ermA*, *ermB*, and *ermC*) gene are sensitive to erythromycin. This difference can be attributed to the lack of expression of these resistance genes.<sup>54</sup>

Recent studies have proved that enterococci from food of animal origin may be related to the colonization and pathogenesis of human diseases.<sup>22</sup> This requires extensive and sustained attention to the encoding and production of multiple virulence factors by potentially pathogenic enterococci. In the current study, the *E. faecalis* isolates exhibited high prevalence of *cpd*, *efaAfs*, *esp* and *gelE*, and the *efaAfs* was the most prevalent virulence gene in *E. faecium*. Similar observations have also been reported by other reports.<sup>2,22</sup> The first step of enterococci infection is colonization in tissues, which is mainly mediated by adhesins anchored by cell walls.<sup>55</sup> In this study, six adhesive virulence genes (*efaAfs*, *efaAfm*, *efaA*, *esp*, *ace*, *asa1*) were detected, except for *efaA* gene, other genes were detected in *E. faecalis* and *E. faecium*. In this study, *efaAfs*, *efaAfm*, *esp*, and *ace* genes were negative in all of *E. hirae*. Recent study also failed to observe *efaAfs* and *efaAfm* genes in *E. hirae* isolates.<sup>56</sup> However, another study observed *efaAfs* and *efaAfm* genes in important foodborne *E. hirae*.<sup>57</sup> This difference may be due to the low prevalence of toxins producing *E. hirae* isolates in the study area. In addition, *E. hirae* may have other untested toxin genes related to bacterial adhesion.<sup>58</sup> The *efaAfm* and *efaAfs* genes are believed to be involved in adhesion to host cells and biofilm formation.<sup>59</sup> The *efaAfm* and *efaAfs* genes were present in a high proportion of *E. faecalis* and *E. faecium* isolates. This study found that 109 strains of *E. faecalis* and 2 strains of *E. faecium* carry the *esp* gene. Previous studies have found *esp* genes contribute to the establishment of successful enterococci infections and increase the colonization and persistence of infections.<sup>60,61</sup> This study found that 43 strains of *E. faecalis* and 2 strains of *E. faecium* carry the *ace* gene. The *ace* gene encodes a collagen adhesin. When the host cells are damaged, the adhesion proteins are exposed, which will promote the adhesion of bacteria to the host cells. Studies have shown that enterococci carrying *ace* gene has stronger adhesion ability.<sup>60</sup> This study found that 38 strains of *E. faecalis*, 8 strains of *E. faecium*, and 2 strains of *E. hirae* carry the *asa1* gene. The *asa1* gene encodes a polymer that binds to extracellular matrix proteins such as fibronectin, thrombospondin, vitronectin, and type I collagen and mediates multiple stages of infection.<sup>62,63</sup> Therefore, we speculate that isolates of *E. faecalis* and *E. faecalis* may have strong initial colonization and biofilm formation abilities.

The *cpd* gene encodes a sex pheromone, a sex pheromone thought to be involved in triggering an inflammatory response.<sup>8</sup> The sex pheromones secreted by bacteria are perceived by potential companion cells, which mediate the contact between bacteria and induce the lateral transfer of the coupling plasmid encoding the determinants of antibiotic resistance and virulence.<sup>64,65</sup> Similar to the results of the present study, *cpd* gene was also found to have the higher detection rate in *E. faecalis* in past studies.<sup>22</sup> However, only two strains of *E. faecalis* were found to carry the *cpd* gene, and all strains of *E. hirae* were negative for the *cpd* gene.

The production of cytolysin has been proved by many experiments to be related to the severity of diseases induced by enterococci.<sup>66,67</sup> Cytolysin has a wide range of target cells, so it is also one of the virulence traits that attract much attention in pathogenic bacteria.<sup>68,69</sup> In this study, we observed the *cylA* gene associated with hemolysin in 16.1% of *E. faecalis* isolates. This result is higher than previous research results.<sup>53,70</sup> The *cylA* gene synthesizes serine protease,



which causes the activation of cytolysin outside the cell.<sup>71</sup> This reaction may cause red blood cell lysis and tissue damage, leading to bacterial transmission within the host and promoting the development of infection. In addition, cytolysin can escape the host immune system by invading and avoiding phagocyte killing.<sup>72</sup>

In addition, we evaluated the gelatinase-related toxin genes. The *gelE* gene was present in 77.4% of *E. faecalis*. This agrees with the results of previous studies on isolated strains of *E. faecium*, they also did not detect the *gelE* gene in *E. faecium*.<sup>8,71,73</sup> Encoded by the chromosomal gene *gelE*, gelatinase is an extracellular zinc endopeptidase that hydrolyzes a variety of proteins or peptides, including casein, hemoglobin, insulin, fibrinogen and collagen.<sup>73</sup> The decomposition of these proteins may contribute to the invasion and spread of pathogenic bacteria to the breast.<sup>74</sup> Therefore, gelatinase is considered to affect the virulence of enterococci and is related to the biofilm formation, infection and spread of enterococci.<sup>75,76</sup> The expression of the *gelE* gene is regulated by a quorum sensing system, which allows the bacterium to coordinate its behavior with other members of its population.<sup>77</sup> When the bacteria reach a certain population density, the quorum sensing system is activated, which leads to the expression of virulence factors including gelatinase.

## Conclusion

This study provided rich species diversity for enterococci causing mastitis in Ningxia. It is worth noting that although the incidence rate of *E. faecalis* is relatively low in clinical bovine mastitis cases, it has an upward trend compared with previous studies. The *E. faecalis*, *E. faecium*, and *E. hirae* displayed high phenotypic and genotypic resistance to tetracycline and erythromycin, which requires more attention through comprehensive monitoring of the antimicrobials use in dairy production. In addition, the high prevalence of *cpd*, *gelE*, *efaAfs*, and *esp* in *E. faecalis* suggests that they may play an important role in causing clinical bovine mastitis in China. Because of the complexity of clinical bovine mastitis caused by enterococci, additional measures must be taken to reduce the development and spread of antimicrobial resistant elements from farms to clinics around the world. In addition, although *E. hirae* carries fewer toxin genes in this study, more extensive studies are needed to prove the true infectivity and pathogenicity of *E. hirae* in mastitis.

## Ethics Approval and Informed Consent

The animal owners were informed about the purpose of the study and consent of each animal owner was obtained before the physical examination of cows for clinical mastitis and the collection of milk samples. All procedures involved in animal care and their use were in strict accordance with the guidelines for the Care and Use of Laboratory Animals, Lanzhou Institute of Husbandry and Pharmaceutical Sciences, CAAS, China (SYXK-2019-0012).

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## Disclosure

The authors report no conflicts of interest in this work.

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