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Correlation Analysis between the Antimicrobial Resistance and Virulence of Pathogenic *Streptococcus* Isolates from Cows

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Y-XD and YM presented the concept. QW and Y-XD planned methodology and curated data. QW and NZ perfromed forrmal analysis. Y-XD wrote the manuscript. YM and NZ reviewed the manuscript. YM acquire funds.

Key words
***Streptococcus*, Induced resistance, Plasmid conjugation, Virulence.**

ABSTRACT

Bovine *Streptococcus* are one of the main pathogens causing bacterial disease such as mastitis and endometritis in dairy farming. The virulence factors produced by *Streptococcus* are related to the occurrence of inflammation. To investigate the correlation between antimicrobial resistance and virulence traits of bovine *Streptococcal* isolates. Induced resistance was conducted for *Streptococcus pneumonia* ATCC49619 and erythromycin-sensitive strains by gradually increasing the antimicrobial concentration. Plasmid conjugation test was carried out by membrane filtration method. The correlation between antimicrobial resistance and virulence traits was analyzed by LD_{50} and related genes. Sensitive *Streptococcus* isolates to erythromycin and *S. pneumoniae* ATCC49619 were induced to resistance *in vitro*, MIC value was from ≤ 0.5 μg/mL up to ≥ 64 μg/mL, and *ermB* or *mefA* resistant gene were carried. Transfer rate of resistance was 100% by plasmid conjugant, conjugants had obtained the resistance phenotype and the related resistance genes from the donor bacteria. The LD_{50} of conjugants and induced resistance strains compared with parental strain, the virulence was lower than sensitive strains. The present study demonstrated that the virulence of resistant *Streptococcus* strains obtained by different drug resistance transfer methods was lower than that of their parents.

INTRODUCTION

Streptococcus belongs to a gram-positive bacterium, which is widely distributed in cow skin, bedding, feces and urine, sewage and other environments. The pathogenic strain can cause cow infection through contact transmission, such as mastitis and endometritis (Wu *et al*., 2019). Additionally, during recent decades, livestock production (including the dairy production) has tended to a high-density and intensive production model, leading to frequent occurrence of animal diseases, and antibiotics are therefore widely used in feed and veterinary clinical practice to prevent and treat animal bacterial infectious diseases (Guo *et al*., 2020; Liu *et al*., 2020), while the rapid emergence and dissemination of resistance has become a major concern of public health security (Liu *et al*., 2019; Wu *et al*., 2019), and it also includes the issue of drug resistance in *Streptococcus*. Besides, in addition to public health issues, drug resistance makes the veterinary clinical treatment of *Streptococcus* extremely difficult and seriously affect the healthy production of dairy cows (Ding *et al*., 2016).

The mechanism of bacterial drug resistance has become an important and extensive research topic in clinical microbiology (Martínez *et al*., 2002). Previous studies have found that the major determinants of the resistant mechanism are derived from horizontal gene transfer in other organisms, and common essential characteristics have also been reported in studies of pathogen virulence. On the other hand, it has been shown that antibiotic resistance genes and virulence genes can be in the same mobile components, such as plasmids, transposons, phages, integrons, and gene clusters (Villa *et al*., 2005; Reid *et al*., 2019). Johnson *et al*. (2005) reported that resistance and virulence genes are located on the same or different plasmids, and both can be transferred simultaneously with plasmid. It was suggested that the plasmid pAPEC-O1-R and ColBM which mediates

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resistance and virulence, respectively, could co-transfer through the conjugation in avian pathogenic *Escherichia coli* (Johnson *et al*., 2006). The pCERC3 plasmid was also found to be both virulence and resistance plasmid in *E. coli* (Moran *et al*., 2016). Further studies have shown a correlation between virulence and antibiotics (Rathnayake *et al*., 2012). Ghorbel *et al*. (2019) observed a significant correlation between the virulence pattern and the map of antimicrobial agents. Azzam *et al*. (2017) showed that multiple antibiotics resistances was strongly correlated with bacterial virulence in wastewater ecosystems. Vila *et al*. (2002) reported that when bacteria acquired antibiotic resistance, their virulence decreased, the study of the mechanism showed that most strains increase resistance by changing their own expression or protein structure (Wang *et al*., 2016).

At present, most studies on its correlation are focused on human medicine and ecological environment, while few studies on bovine *Streptococcus*. Hence, the present study was conducted to investigate the correlation between antimicrobial resistance and virulence traits of bovine *Streptococcal* isolates by using *in vitro*-induced drug resistance and resistant plasmid conjugation of sensitive *Streptococcal* isolates, which will render rationale basement for controlling bacterial disease caused by *Streptococcal* infection.

MATERIALS AND METHODS

Tested strains

S. pneumoniae ATCC49619 was provided by laboratory. Bovine *Streptococcal* isolates which sensitive to erythromycin (MIC < 1 μg/mL) and the donor bacteria both came from clinical cases. *Streptococcus dysgalactiae* CVCC3701 and *Streptococcus dysgalactiae* CVCC3701-PEN (penicillin-induced resistance) were provided by other researchers in this study.

In vitro *induced resistance test*

The preserved standard strain (ATCC49619) and 15 erythromycin-sensitive *Streptococcal* isolates were inoculated into the BHI broth with serum, and incubated at 37℃ for 6 h. A small amount of bacterial solution was picked to mark on M-H agar plates and incubated at 37℃ for 16-20 h. BHI broth containing the sub inhibitory concentration of antibiotic was prepared and passed at 37℃. Meanwhile, the negative control of broth was made and transferred every 3 days. The concentration of the induced antibiotic was gradually increased by 2 times until the MIC of the test strain rose to the resistance range (Gautier *et al*., 2002). The stability of resistant progeny was tested, and related resistant genes (*ermB*, *mefA*) were detected by PCR. Primer information was shown in Table I.

Plasmid conjugation test

The test was performed by membrane filtration method (Werner *et al*., 2003). 20 *Streptococcal* isolates that were both resistant to tetracycline and sensitive to erythromycin were selected as the donor bacteria, and *S. pneumoniae* ATCC49619-ERY (erythromycin-induced resistance) was used as the recipient bacteria. The suitable antibiotic concentrations of erythromycin and tetracycline were screened, respectively. The tested strains were inoculated into the BHI broth with serum and incubated at 37℃ for 18 h aerobically in 5% CO_2. That was adjusted to 10^8 CFU/mL. The 5 μL bacteria solution was absorbed

Table I.- Details of PCR primers.

Target gene	Primer sequence (5'–3')		Tm. (°C)	Amplicon size (bp)	Reference
	Forward	Reverse			
ermB	ATTGGAACAGGTAAAGGGC	GAACATCTGTGGTATGGCG	50	442	Marimo´n *et al*. (2005)
mefA	AGTATCATTAATCACTAGTGC	TTCTTCTGGTACTAAAAGTGG	53	346	Marimo´n *et al*. (2005)
tetM	GAACTCGAACAAGAGGAAAGC	ATGGAAGCCCAGAAAGGAT	50	993	Lopardo *et al*. (2003)
tetL	TGAACGTCTCATTACCTG	ACGAAAGCCCACCTAAAA	50	189	Lopardo *et al*. (2003)
bac	TGTAAAGGACGATAGTGTGAAGAC	CATTTGTGATTCCCTTTTGC	50	530	Dmitriev *et al*. (2002)
bca	TAACAGTTATGATACTTCACAGAC	ACGACTTTCTTCCGTCCACTTAGG	51	535	Dmitriev *et al*. (2002)
scpB	CCAAGACTTCAGCCACAAGG	CAATTCCAGCCAATAGCAGC	57	591	Dmitriev *et al*. (2002)
lmb	ACCGTCTGAAATGATGTGG	GATTGACGTTGTCTTCTGC	51	572	Dmitriev *et al*. (2002)
cyl	ACGGCTTGTCCATAGTAGTGTTTG	AACGACACTGCCATCAGCAC	52	345	Dmitriev *et al*. (2002)
glnA	ACGTATGAACAGAGTTGGCTATAA	TCCTCTGATAATTGCATTCCAC	52	471	Dmitriev *et al*. (2002)
cfb	ATGGGATTTGGGATAACTAAGCTAG	AGCGTGTATTCCAGATTTCCTTAT	52	193	Dmitriev *et al*. (2002)
hylB	ACAAATGGAACGACGTGACTAT	CACCAATTGGCAGAGCCT	52	346	Dmitriev *et al*. (2002)

into 1 mL liquid medium for 4 h by shaking culture. The donor and recipient were mixed at 1:3 (20 μL, 60 μL) and coated on the sterile filter membrane. The filter membrane was placed in the BHI agar plates at 37℃ for 18-24 h, after that was washed with 1 mL BHI broth and transplanted into BHI agar plates containing a certain concentration of erythromycin and tetracycline. The results were observed after 36-48 h. Single colony was selected and inoculated into LB broth at 37℃ for the identification of conjugants.

Conjugant identification

MIC and multiple PCR were used to identify the conjugants (Huys *et al*., 2004). MIC was detected by double dilution method.

Detection of virulence on bovine Streptococcus

LD_{50} assay was performed on strains with resistant phenotypes, resistant and virulence genes (*bac, bca, scpB, LMB, cyl, glnA, CFB, hylB*) after induction and plasmid conjugation. Half of the lethal dose (LD_{50}) was determined by Bliss (1936) method. The experimental design was divided into a blank control group and six experimental groups. Three dilution degrees were set in equal ratio between the LD_0 and LD_{100}, with a total of 5 gradients in the

Table II.- MIC results of sensitive *Streptococcus* induced resistance by erythromycin.

Group	Strain	Species	MIC (μg/mL)		Induction algebra
			Before induction	After induction	
Blank control	ATCC49619	*S. pneumoniae*	0.25	0.25	—
Negative control	ATCC49619	*S. pneumoniae*	0.25	0.25	—
Experimental group	ATCC49619	*S. pneumoniae*	0.25	256	10
	FL1	*S. agalactiae*	0.12	128	12
	FL2	*S. agalactiae*	0.5	128	12
	FL3	*S. agalactiae*	0.25	128	10
	FL4	*S. agalactiae*	0.25	128	10
	FL5	*S. agalactiae*	0.12	64	10
	FL6	*S. agalactiae*	0.25	64	10
	FL7	*S. agalactiae*	0.5	256	12
	FL8	*S. agalactiae*	0.5	256	12
	FL9	*S. agalactiae*	0.25	256	10
	FL10	*S. agalactiae*	0.12	128	12
	FL11	*S. agalactiae*	0.12	64	10
	FL12	*S. agalactiae*	0.12	128	12
	FL13	*S. dysgalactiae*	0.25	256	10
	FL14	*S. dysgalactiae*	0.25	128	10
	FL15	*S. uberis*	0.5	128	10

Table III.- The MIC of conjugants, donor and receptor bacteria against antimicrobial agents.

Antimicrobial agents	MIC(μg/mL)				
	Donor bacteria		Receptor bacteria (ATCC49619-ERY)	Conjugons	
	MIC_{50}	MIC_{90}	MIC	MIC_{50}	MIC_{90}
Penicillin	16	128	0.125	1	8
Ampicillin	0.5	1	0.25	0.25	1
Amoxicillin	2	16	0.125	2	32
Erythromycin	0.25	0.5	256	>256	>256
Chloramphenicol	8	32	2	1	64
Ofloxacin	1	8	1	0.5	1
Levofloxacin	0.25	2	0.063	0.125	0.25
Tetracycline	64	128	1	64	128
Clindamycin	8	32	0.125	16	64
Vancomycin	1	4	0.25	1	2
Kanamycin	64	256	4	128	128

MIC_{50} value is the MIC value that inhibited at least 50% of the isolates, MIC_{90} value is the MIC value that inhibited at least 90% of the isolates.

experimental group. After inoculation, the symptoms, time of death and the number of deaths were recorded.

RESULTS

Erythromycin-sensitive strain induced resistance

MIC values of all strains after erythromycin induction were shown in Table II. The results showed that 15 clinical isolates and *S.pneumoniae* ATCC49619 developed high resistance after induction for more than 10 generations (MIC ≥ 64 μg/mL). MIC value of *S.pneumoniae* ATCC49619 reached 256 μg/mL. Only 3 *S.* agalactiae had been 64 μg/mL after induction, and other isolates had MIC ≥ 128 μg/mL. The MIC value of resistant offspring was not changed after culturing in a drug-free medium for 5 generations. The resistance was stable.

Antimicrobial resistance genes

Resistant genes were detected by PCR after inducing. 15 strains amplified a fragment of 442bp (*ermB*), and 1 strain amplified a fragment of 346bp (*mefA*). Partial test results were shown in Figure 1.

Plasmid conjugation test

Plasmid conjugation was performed between 20 donor and the recipient bacteria (ATCC49619-ERY), respectively. Conjugation and parent strains were also tested for their susceptibility to 11 antimicrobial agents and the results are shown in Table III. The MIC value of the recipient bacteria against erythromycin was 256 μg/mL. At least 50% of the donor bacteria showed resistance to tetracycline (MIC_{50} value = 64 μg/mL and MIC_{90} value = 128 μg/mL). The MIC_{50} and MIC_{90} values of conjugation against erythromycin were greater than 256 μg/mL, MIC_{50} to tetracycline was 64 μg/mL, and MIC_{90} was 128 μg/mL after plasmid conjugation test. In addition, the resistant phenotypes of β-lactams, quinolones, amides and vancomycin did not transfer to the conjugation, while the resistant phenotypes of lincomines in the donor bacteria were more consistent with conjugation.

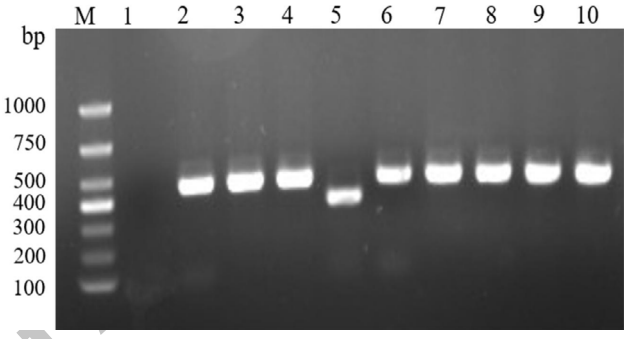

Fig. 1. Distribution of erythromycin resistant genes by induced-resistance. M, 1000DL marker; 1, Blank; 2, *S. pneumoniae*; 3-4 and 6-8, *S. agalactiae*, ermB gene; 5, *S. agalactiae*, mefA gene; 9, *S. dysgalactiae*, ermB gene; 10, *S. uberis*, ermB gene.

Table IV. Distribution of virulence genes and resistance in tested strains.

	Tested strains				
	Donor bacteria WR38	**Receptor bacteria ATCC49619-ERY**	**Conjugon PC12**	***S. dysgalactiae* CVCC3701**	***S. dysgalactiae* CVCC3701-PEN**
R-phenotype	TET^+, PEN^+	ERY^+	TET^+, ERY^+	—	PEN^+
Resistance genes					
tetM	+	-	+	-	-
tetL	+	-	+	-	-
ermB	-	+	+	-	-
mefA	-	-	-	-	-
Pbp1a	-	-	-	-	+
pbp2b	-	-	-	-	+
Virulence genes					
bac	-	-	-	-	+
bca	-	-	-	-	+
scpB	-	-	-	-	+
lmb	-	-	-	-	+
cyl	+	-	-	+	+
glnA	+	-	-	-	+
cfb	+	-	-	-	+
hylB	+	-	-	-	+

Identification of conjugons

The DNA of conjugation was extracted and multiplex PCR was performed. Partial electrophoresis results are shown in Figure 2. The results showed that the tetracycline resistance genes *tetM* (740bp) and *tetL* (993bp) be detected in the donor bacteria with high tetracycline resistance, *ermB* (442bp) be detected in the recipient bacteria with high erythromycin resistance, and both tetracycline and erythromycin resistance genes be detected in the conjugation.

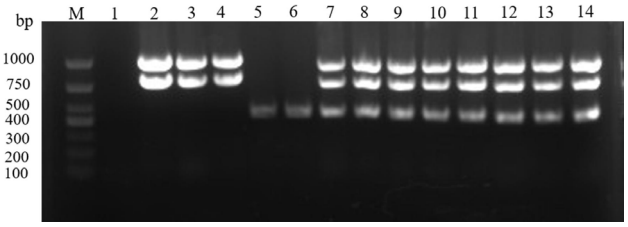

Fig. 2. Resistance genes of conjugants, donor and receptor after plasmid conjugation. M, 1000DL marker; 1, black; 2-4, donor (tetM and tetL of *S. agalactiae* and *S. dysgalactiae*); 5-6, receptor (ermB of ATCC49619-ERY); 7-14, conjugants (ermB, tetM, tetL).

Table V.- LD_0 and LD_{100} of tested strains.

Tested Strains	Lethal dose (CFU/mL)	
	LD_0	LD_{100}
Donor bacteria WR38	4.9×10^6	4.9×10^{10}
Receptor bacteria ATCC49619-ERY	2.6×10^5	2.6×10^9
Conjugon PC12	2.0×10^6	1.6×10^8
S. dysgalactiae CVCC3701	4.0×10^5	1.0×10^8
S. dysgalactiae CVCC3701-PEN	1.4×10^8	1.4×10^{10}

Virulence of Streptococcus

LD_{50} was performed on 5 strains of induced resistance and plasmid conjugation. The genes of resistance and virulence are shown in Table IV. According to the preliminary experimental results, the LD_0 and LD_{100} of all the tested strains are shown in Table V, and the LD_{50} results are shown in Table VI. The LD_{50} of donor bacteria (name: WR38), recipient bacteria (name: ATCC49619-ERY) and conjugation (name: PC12) from plasmid conjugation transfer test were analyzed by Bliss method, the results showed that the virulence of conjugation PC12 was decreased compared with that of the recipient ATCC49619-ERY, and its LD_{50} was increased by 1.6 times compared with that of the recipient.

The results of CVCC3701 and CVCC3701-PEN of *S. dysgalactiae* from the induced resistance test showed that the LD_{50} value of the strain was significantly different before and after induction, and the LD_{50} of the strain after induction was increased by 1.1×10^2 times compared with that before, indicating that the virulence of the strain after induction decreased significantly.

Table VI.- The LD_{50} of tested strains on the mouse. A dose (0.5 mL) of vaccine was administered to 8 mice i.p. for each experimental group.

Tested dtrains	Group	Concentration (CFU/mL)	Deaths	LD_{50} (CFU/mL)
Sterile LB broth	Blank control	—	0	—
Donor bacteria WR38	1-LD_{100}	4.9×10^{10}	8	1.13×10^8
	1-$n_1{}^3LD_0$	4.9×10^9	5	
	1-$n_1{}^2LD_0$	4.9×10^8	2	
	1-n_1LD_0	4.9×10^7	1	
	1-LD_0	4.9×10^6	0	
Receptor bacteria ATCC49619-ERY	2-LD_{100}	2.6×10^9	8	5.41×10^6
	2-$n_2{}^3LD_0$	2.6×10^8	7	
	2-$n_2{}^2LD_0$	2.6×10^7	3	
	2-n_2LD_0	2.6×10^6	2	
	2-LD_0	2.6×10^5	0	
Conjugon PC12	3-LD_{100}	1.6×10^8	8	8.92×10^6
	3-$n_3{}^3LD_0$	5.3×10^7	4	
	3-$n_3{}^2LD_0$	1.7×10^7	2	
	3-n_3LD_0	6.0×10^6	1	
	3-LD_0	2.0×10^6	0	
S. dysgalactiae CVCC3701	4-LD_{100}	1.0×10^9	8	5.45×10^6
	4-$n_4{}^3LD_0$	1.4×10^8	7	
	4-$n_4{}^2LD_0$	1.98×10^7	3	
	4-n_4LD_0	2.8×10^6	1	
	4-LD_0	4.0×10^5	0	
S. dysgalactiae CVCC3701-PEN	5-LD_{100}	1.4×10^{10}	8	5.82×10^8
	5-$n_5{}^3LD_0$	4.4×10^9	7	
	5-$n_5{}^2LD_0$	1.1×10^9	6	
	5-n_5LD_0	4.4×10^8	3	
	5-LD_0	1.4×10^8	0	

DISCUSSION

Erythromycin-sensitive Streptococcus *induced resistance*

Streptococcus as the main pathogens that cause a variety of suppurative inflammation in animals and humans, such as mastitis, endometritis, sepsis and neonatal sepsis, meningitis. Macrolides are a class of antibiotics

used in the treatment of gram-positive bacterial infections. Some of them are also added into the feed, resulting in the gradual increase of resistance.

At present, the resistance rate of *S. agalactiae* to erythromycin in mastitis was as high as 94.1% from some parts of China, while India was 33.3% (Jain *et al*., 2012). Previous studies also found that the resistance rate of group B *Streptococcus* against erythromycin was also increasing by years in Canada and Taiwan (Sherman *et al*., 2012; Ko *et al*., 2001; Helena *et al*., 1997). The resistance rate of *Streptococcus suis* to macrolides was more than 50% (Martel *et al*., 2001), In Asia, such as China, Vietnam and Korea, clinical isolates of *S. pneumoniae* had resistance rates of over 70% to macrolides (Song *et al*., 2004; Sahm *et al*., 2008). Erythromycin resistance model was successfully established by induction *in vitro*, and the adaptability of *Streptococcus* was proved to be different due to antibiotic differences in the experiment. *ErmB* gene was detected in most resistant *Streptococcus* in the test, suggesting that erythromycin resistance methylase may be the major mechanism of resistance in the present study. The results suggest that low doses and concentrations of drugs will not kill bacteria, but it will adapt to the environment by producing the corresponding resistance genes or genetic mutations to escape clinically. The standard strain ATCC49619-ERY of *S. pneumoniae* resistant only to erythromycin was obtained through the model, and the changes in virulence characteristics of the same strain before and after induction of resistance could be more directly compared, which provided a single resistant strain for subsequent plasmid conjugating tests.

Plasmid conjugation test of bovine Streptococcus

Bacterial resistance includes intrinsic and acquired resistance. Studies have shown that many resistant genes are located on mobile DNA components such as plasmids, transposons and integrons, and can also be transmitted between bacteria by conjugation plasmids, transposons, integrons and phages (Zhao *et al*., 2011). The acquisition of resistant plasmids is the most common mechanism of bacterial resistance (Bruinsma *et al*., 2004), conjugation is the primary mode of transmission of resistant genes in bacteria (Brown *et al*., 1999).

In this study, a high tetracycline-resistant *Streptococcal* plasmid conjugation and transfer test was carried out by membrane grafting method. The resistance of tetracycline and related resistance genes (*tetM* and *tetL*) were successfully transferred into the recipient bacteria by 20 strains of highly tetracycline resistant donor. More than 80% of the colonies growing in the two antibiotics screening plate acquired the properties of the donor and recipient bacteria, while the remaining colonies only acquired the resistance phenotype of tetracycline but did not detect genes. The two antibiotics concentration was selected for screening to determine the conjugation and distinguish from donor and recipient bacteria.

The resistance to tetracycline is mainly acquired through the resistance gene transfering in conjugation plasmids, and the *tetL* gene encodes efflux pump protein which often exists in small plasmids with transmissibility. *TetM* gene encodes ribosomal protective proteins which mainly located in transposons of the Tn916-Tn1545 family (Huys *et al*., 2004). The family forms a ring structure, which can transfer intracellular and intercellular. Tn916 has a wide host range and can transfer the *tetM* gene to gram-negative bacteria and even to mycoplasma (Lancaster *et al*., 2004), which may be the reason why tetracycline is susceptible to transfer to others. It is worth noting that the MIC of clindamycin in conjugation is improved compared with the recipient bacteria. Lincomines can be methylated by ribosomes, and produced cross-resistant with macrolidenes. The macrolidene resistance gene *ermB* is usually located in Tn1545 and Tn917 (Okitsu *et al*., 2005), suggesting that the transfer of resistance of lincomines may be related to transposon Tn1545.

LD50 of bovine Streptococcus *before and after resistance*

In order to demonstrate the changes in resistance and virulence characteristics of *Streptococcus*, we used plasmid conjugation and *in vitro* induced resistance tests to gradually transform the sensitive into resistant *Streptococcus* with clear background to demonstrate the changes of the same strain in the process of resistance. The results showed that the LD_{50} value of the inducted strain CVCC3701-PEN increased by 1.1×10^2 times compared with that of the parent strain before induction. the LD_{50} value of conjugation increased by 1.6 times compared with that of the recipient bacteria, indicating that the virulence of the sensitive bacteria decreased after acquiring resistance. Some researchers had found that virulence and resistance genes can be transmitted and transformed to a certain extent (Alexander *et al*., 2011; Barton, 2000), antibiotic resistance also existed in genes that encode bacteriocins (Chelliah *et al*., 2019), iron carriers (Zhang *et al*., 2017), cytotoxins (Carlson *et al*., 2001), and adhesion factors (Laporta *et al*., 1986).

Pathogenicity islands (PAI) is a special genomic island that contains multiple virulence genes as well as plasmids, transposons and integrons. PAI constructed a new genomic island by horizontal transfer of virulence genes through plasmids or transposons, thereby expanding the bacterial spectrum of the virulence island. However, PAI was often associated with a tRNA gene or insertion sequence, and present in strong strains generally, but rarely distributed

in the associated weak or no strains (Zhu *et al*., 2013). It is suggested that the donor bacteria may not exist a PAI due to its weak virulence (LD_{50} = 1.13×10^8 CFU/mL), and virulence factors are difficult to transfer horizontally.

It has demonstrated that penicillin-resistant *S. pneumoniae* may be less virulent than sensitive strains (Azoulay *et al*., 2000), multiple drug resistance leading to reduced virulence (Lehtolainen *et al*., 2003). Resistance to rifampicin leaded to a decrease in virulence (Neill *et al*., 2006). The expression of virulence genes was decreased in fluoroquinolone-resistant strains (Schaeffer, 2002; Liu *et al*., 2009). In this regard, many scholars put forward the concept of biological adaptability cost, the decrease of fitness caused by resistance mutation (Andersson, 2006). Therefore, abnormal bacterial regulation may occur when movable elements are engaged, and that is the increased cost of adaptation. Björkman *et al*. (1998) reported that resistant *Salmonella typhimurium* was less virulent to mice due to mutations in *rpsL*, *rpoB* and *gyrA* genes, but it quickly repaired its adaptability and virulence by compensating mutations. It is suggested that the toxicity of resistant bacteria decreased, while a variety of virulence genes were detected after induction of resistance, which may be caused by the fact that *S. dysgalactiae* CVCC3701-PEN stimulated compensatory adaptation, that needs to be further verified.

CONCLUSION

To summarize, based on the findings of the above three experiments, this study applied the standard strain of *S. pneumoniae* and *S. dysgalactia*e, and obtained different resistant strains with different drug resistance transfer methods, the results that the virulence of the resistant strain decreased compared with the parent strain, so that the results were mutually supported and verified.

Data availability statement

All public data generated or analyzed during this study are included in this article. Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Statement of conflict of interest

The authors report that they have no conflicts of interest.

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