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Relationship Between Fluoroquinolone Resistance and *gyrA*, *parC* Gene in *Streptococcus suis* Type 2 Isolates in Hebei Province China

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Abstract: To detect the relationship between fluoroquinolone resistance and *gyrA*, *parC* gene mutation in *Streptococcus suis* type 2. The MIC of 14 strains of *Streptococcus suis* type 2 were determined by microdilution. The genes encoding the Quinolone-Resistance Determining Region (QRDRs) of parC and gyrA in fluoroquinolone-susceptible and resistant *Streptococcus suis* clinical isolates were identified and sequenced. Ser to Phe and Arg to Leu mutation at position 79 and 87 of the *parC* gene was detected in fluoroquinolone resistance SS2 and Arg to Ser or Ser to Arg mutations at the position 66 or 81 of *gyrA* gene were detected in 2 highly resistance strains; no amino acid changes in gyrA or parC were detected for 7 fluoroquinolone-susceptible strains; the mutations in both genes were found in the strains with MIC of fluoroquinolone >32 μg mL^{-1}. Mutations in *parC* gene may result in low level resistance against fluoroquinolone and mutations in both *gyrA* and *parC* genes result in high level resistance.

Key words: *Streptococcus suis*, fluoroquinolone, *parC* gene, *gyrA* gene, amino acid, China

INTRODUCTION

Streptococcus suis, a class of swine diseases caused by streptococcal bacteria has occurred all over the world. Some pathogenic streptococci are important zoonotic pathogens that can lead to a variety of suppurative diseases, pneumonia, meningitis, endocarditis, septicemia and septic arthritis which caused great harm to human and animal health (Kongwang and Chengping, 2000; Najati and Saboktakin, 2010; Seyed *et al*., 2011). Its high morbidity and mortality caused severe economic losses to the porcine industry. In recent years with the inappropriate use of antibiotics, *Streptococcus suis* is also experienced varying degrees of antibiotic resistance including fluoroquinolone resistance. At present, it is only reported that *parC*, *gyrA* gene mutations of *Streptococcus suis* separated from of the Northeast region of China associate with drug resistance (Yuan *et al*., 2009). Nucleotide and amino acid mutations of *parC*, *gyrA* gene of fluoroquinolone-resistant *Streptococcus suis* separated from Hebei province of China were deteced on this study and the relationship between these mutations in *parC*, *gyrA* gene and the different degrees of resistance was explained.

MATERIALS AND METHODS

Origin of strain: The *Streptococcus suis* type 2 strains were separated from lungs, spleen, kidney, heart, brain and synovial fluid in pigs with typical infection of *Streptococcus suis* in Qinhuangdao, Tangshan, Zhangjiakou, Shijiazhuang, Handan of Hebei province during 2005-2010. About 14 strains were obtained by PCR identification. *Staphylococcus aureus* ATCC29213 as the quality control strain were purchased from Hangzhou Tianhe Microorganism Reagent Co., Ltd.

Medium and reagents: MH mediums were purchased from Hangzhou Tianhe Microorganism Reagent Co., Ltd. and 5% calf serum was added to it before usage; drug standards were purchased from China Institute of Veterinary Drugs Control; Taq DNA Polymerase (plus) (2 U μL^{-1}), DNA Marker DL2000 (60 ng μL^{-1}) were from TaKaRa Biotechnology Co., Ltd. Goldview dye, centrifugal cylindrical agarose gel, DNA purification kit Dye, Agarose Gel DNA Extraction kit (Centrifugal columnar) purchased from Tiangen Biotech (Beijing) Co., Ltd.

Primers: Primers were designed accroding to the reference (Escudero *et al*., 2007). The primers of gyrA were 5'-CGCCGTATTTTGTATGGGATG-3' and 5'-GTTCCGTTAACCAGAAGGTT-3'. The primers of parC were 5'-AAGGACGGCAACACTTTTGAC-3' and 5'-AGTGGGTTCTTTTTCCGTATC-3'. Product length was 377 and 312 bp while annealing temperature 48.9 and 42.7°C, respectively. The fragments were amplified and then sequenced Sangon Biotech (Shanghai) Co., Ltd.

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Drug sensitivity test *in vitro*: About 14 strains of SS2 were measured the Minimum Inhibitory Concentration (MIC) to 4 fluoroquinolones including Ciprofloxacin (CPF), Enrofloxacin (ENR), Ofloxacin (OFL) and Levofloxacin (LVX). The specific operation and the results were according to NCCLS standards (M31-A2. Wayne, USA. 2000) (National Committee for Clinical Laboartory Standards, 2000).

Preparation of PCR template: About 7 resistant strains and 7 susceptible strains were selectd. The colonies on rabbit blood agar plates were transferred to a containing 5% calf serum Streptococcus medium and grown at 37°C overnight. The 1 mL preparation in EP tube was centrifuged for 1 min at 12,000 rpm to remove supernatant the cellular debris, then resuspended in 200 μL deionized water. After boiled for 10 min, the strains were centrifuged for 3 min at 12000 rpm. Finally, the supernatant without precipitation were stored in -20°C as the PCR template.

***gyrA*, *parC* gene detection:** Amplification was performed in a total volume of 25 μL containing 2.0 μL dNTP mixture (each 2.5 mM), 1.0 μL each primer (10 pmol μL^{-1}), 0.5 μL ExTaq DNA polymerase (1.25 U), 2.5 μL 10×PCR buffer (Mg_2+Plus), 2.5 μL DNA template and distilled water. The PCR assay comprised preincubation at 94°C for 5 min followed by 30 cycles of 94°C 1 min, 48.9/42.7°C 50 sec and 72°C 1 min. A final extension was performed at 72°C for 10 min. The PCR products were visualized by electrophoresis on a 1% agarose gel by standard procedures.

***gyrA*, *parC* gene recovery and sequencing:** The PCR products, 312 bp *gyrA* gene fragment and 377 bp *parC* gene fragment, purified by gel, then were recovered and sequenced. Sequencing was performed by Sangon Biotech (Shanghai) Co., Ltd. and the sequence data were analyzed using BLAST of GenBank and BioEdit Software.

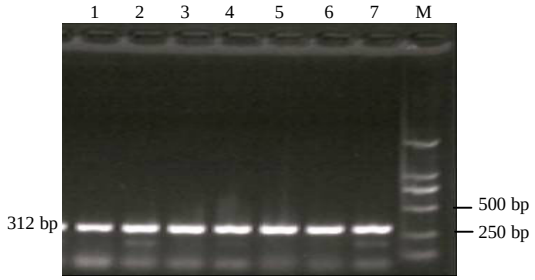
RESULTS AND DISCUSSION

Fluoroquinolones against strains of *S. suis* isolated antibacterial activities *in vitro*: For selected ciprofloxacin, ofloxacin, enrofloxacin and levofloxacin four fluoroquinolone antibacterial drugs, 14 strains of SS2 isolates inhibitory rate were 50 (7/14), 50 (7/14), 50 (7/14) and 57.1% (8/14), respectively. The MIC values of 7 highly resistance strains and 7 susceptible strains were shown in Table 1.

Reasults of *parC* and *gyrA* gene amplification: About 14 strains of SS2 isolates were amplified using chromosomal DNA as temple, the fragment of *parC* and *gyrA* gene were deteced , consistence with the expected size of 312 (Fig. 1) and 377bp (Fig. 2).

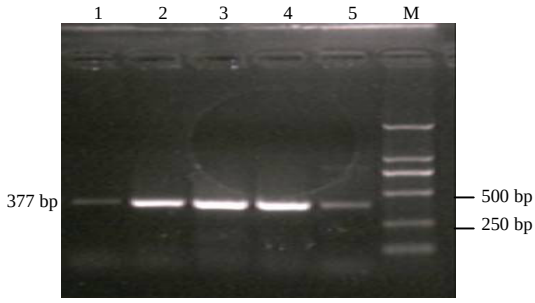
Table 1: Activities *in vitro* of selected fluoroquinolones for SS2 isolated

	MIC (μg mL^{-1})			
Strains	CPF	OFL	ENR	LVX
1	256.000	256.0	128.00	128.00
2	128.000	128.0	64.00	64.00
3	16.000	16.0	16.00	8.00
4	32.000	16.0	16.00	16.00
5	32.000	16.0	32.00	16.00
6	64.000	32.0	16.00	16.00
7	16.000	8.0	8.00	1.00
8	0.250	0.5	0.50	0.50
9	0.125	0.5	0.25	0.25
10	0.500	1.0	0.50	1.00
11	0.500	1.0	0.50	1.00
12	0.500	1.0	0.50	1.00
13	0.500	1.0	0.50	0.50
14	0.500	1.0	0.50	1.00



1-7: parC positive strain; M: Maker

Fig. 1: PCR amplification of parC of chromosomal DNA in SS2



1-5: gyrA positive strain; M: Maker

Fig. 2: PCR amplification of gyrA of chromosomal DNA in SS2

The amino acid sequence analysis of *parC* gene: Compared with the reference sequence AB287006.1, the mutations on the amino acid sequence of parC about 7 drug-resistant strains are less. There are 2 strains with Ser79→Phe, 1 strains with Arg87→Leu in the Quinolone Resistance-Determining Regions (QRDRs) of the *parC* gene in streptococci and 3 strains occurred Met126→Val beyond the QRDR. The other one is no mutations (Fig. 3).

The amino acid sequence analysis of *gyrA* gene: Compared with the reference sequence AB081330.1.,

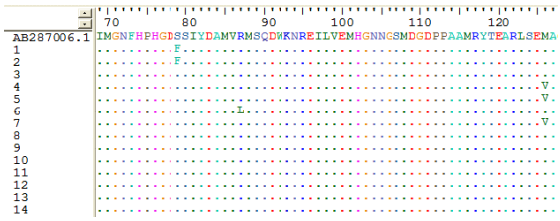


Fig. 3: Comparison of amino acid sequence of parC (fragments involving mutational sites)

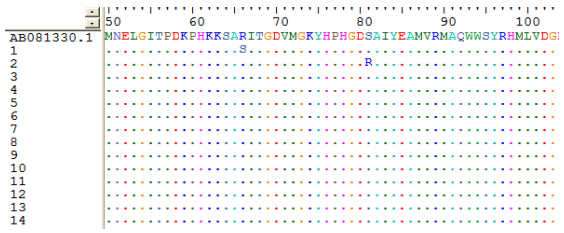


Fig. 4: Comparison of amino acid sequence of gyrA (fragments involving mutational sites)

result were showed. One strains presented Arg66→Ser, 1 strains presented Ser81→Arg in the Quinolone Resistance-Determining Regions (QRDRs) of the *gyrA* gene in streptococci. There were six resistant strains without mutations being found (Fig. 4).

From the sensitivity test results, 14 strains of *Streptococcus suis* isolates showed significant cross-resistance to ciprofloxacin, enrofloxacin, ofloxacin and levofloxacin. The resistance rate was 42.9~50%. The number of high-leval resistant strains is increasing year by year. With the rapid development of economy and the increaseing use of antibiotics in the prevention of swine diseases, resistance of *Streptococcus suis* has been so widespread and severe, researchers should pay more attention to the clinical treatment process.

The mutations in amino acid sequence of *gyrA* gene about 14 strains of *Streptococcus suis* are not much. In the Quinolone Resistance-Determining Regions (QRDRs) of the *gyrA* gene in *Streptococcus suis* Arg66→Ser and Ser81→Arg in the study which are inconsistent with the previous report (Yuan *et al*., 2009, Escudero *et al*., 2007) with Ser81→Lys, Ser81→Phe, Ser81→Tyr, Ser81→Ile in foreign reports and Ser81→Val, Gln→Arg. These two mutations have not been reported. There are 2 strains with Ser79→Phe, 1 strains with Arg87→Leu in the Quinolone Resistance-Determining Regions (QRDRs) of the *parC* gene in 7 resistant strains of streptococci and 3 strains presented Met126→Val beyond the QRDR. No mutation in 1 resistant strain was found. Ser79→Phe is consistent with reports (Escudero *et al*., 2007) and differs from Ser80, Ile 70, G1y78 and Leu89 reported (Yuan *et al*., 2009).

CONCLUSION

This study shows that it was found that the QRDR of *parC* and *gyrA* gene were detected no mutations when *Streptococcus suis* had MICs to ciprofloxacin and enrofloxacin of <1 μg mL^{-1} to Ofloxacin and levofloxacin of <2 μg mL^{-1}. With MICs of ≥8 μg mL^{-1}, mutations Ser79→Phe, Arg 87→Leu, Met 126→Val were presented in *parC* gene, of which Ser79→Phe is the most common. The strains with MICs of 32 μg mL^{-1} presented Ser79→Phe in ParC subunite and Arg66→Ser, Ser81→Arg in gyrA subunite. It was confirmed that the changes of single topoisomerase IV caused low-level resistance to quinolones in *Streptococcus suis* while the high-level resistance is the result of the changes of topoisomerase IV and DNA gyrase (Davies *et al*., 1999; Varon *et al*., 1999). It was also shown that some drug-resistant strains were no found amino acid mutations in *parC* and *gyrA* genes. Whether fluoroquinolone resistance in *Streptococcus suis* was caused by efflux mechanism (Wang *et al*., 2007) and plasmid-mediated horizontal transmission mechanism (Hopkins *et al*., 2007) or not still for further study.

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