Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

# Dual Efflux Pumps SatA and SatB Are Associated with Ciprofloxacin Resistance in *Streptococcus suis* Isolates

Qian Xiaolu[1], Shang Kexin[1], Jam Kashif[1, 2], Huang Jinhu[1] and Wang Liping[1]*

[1]College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu Province, 210095, PR China
[2]Sindh Agriculture University, Tandojam 70060, Pakistan
*Corresponding author: wlp71@163.com

ARTICLE HISTORY (13-441)

Received: September 25, 2013
Revised: April 09, 2014
Accepted: May 18, 2014

**Key words:**
ABC transporters
Efflux pump
Fluoroquinolone resistance
*S suis*

**A B S T R A C T**

*Streptococcus Suis* is an important zoonotic pathogen and is gaining attention due to emergence of drug resistance and recently reported some deaths of human by this pathogen. Recently, fluoroquinolone (FQ) resistant strains of *Streptococcus suis* in animal as well as in human clinics are increasingly reported worldwide. Up to now no study on role of efflux pumps in FQs resistance has been documented, therefore we analyzed resistance mechanisms for FQs in stepwise induced mutants of *S. suis* strains. Results showed some resistant strains without alterations within QRDR of DNA gyrase enzyme and topoisomerase IV but with a FQs-resistant phenotype. MIC of ciprofloxacin, not enrofloxacin against resistant isolates can be reduced by adding reserpine. It suggests that there were any efflux pumps contributed to ciprofloxacin resistance in *S. suis*. Furthermore, growth inhibition assays and its parallel assays were performed and the results intensively indicated there are any efflux pumps in ciprofloxacin resistant strains. Based on the high homology of SatA, SatB and SmrA with PatA, PatB and PmrA, which mediated resistance to FQs in *Streptococcus pneumococcus*, thus the mRNA expression level of *satA*, *saB* and *smrA*were investigated. Overexpression of *satA* and *satB* was found in ciprofloxacin-resistant isolates but expression levels of *smrA* were not significantly changed in resistant strains if compared with their parental sensitive strains. In addition, isolates overexpressing *satA* and *satB* accumulate significantly less ciprofloxacin. In conclusion, all these data represent that SatA and SatB, not SmrA play a clinically relevant role in ciprofloxacin resistance.

©2014 PVJ. All rights reserved

**To Cite This Article:** Xiaolu Q, S Kexin, J Kashif, H Jinhu and W Liping, 2014. Dual efflux pumps satA and satB are associated with ciprofloxacin resistance in *Streptococcus suis* isolates. Pak Vet J, 34(4): 438-443.

## INTRODUCTION

*S. suis* is the major cause for meningitis, pneumonia, endocarditis, arthritis, septicemia or even acute death in animals and recently it has emerged as a zoonotic pathogen and caused similar infections in humans even amongst those who have no history of being in contact with swine or pork (Suankratay *et al.*, 2004; Huang *et al.*, 2005; Tang *et al.*, 2006; Yu *et al.*, 2006; Gottschalk *et al.*, 2010; Kerdsin *et al.*, 2011). Due to the lack of available commercialized vaccines, antibiotic treatment remains to be the main pathway to cure infection of *S. suis* (Higgins and Gottschalk, 2006; Feldman and Anderson, 2011; Hussain *et al*., 2012). Use of beta-lactams, macrolides and FQs is increasing since last decade and studies have shown that various bacterial species including *S. suis* strains have been found multidrug resistant particularly to these families of antimicrobials and the resistant strains are disseminated worldwide.

It is generally considered that the substitutions of QRDR in GyrA subunit of DNA gyrase and ParC subunit of topoisomerase IV were the main causes for high-level FQ resistance in *S. suis*. Over expression of PatA and PatB is confirmed to confer low resistance to FQs in *Streptococcus pneumonia* (Robertson *et al.*, 2005; Marrer *et al.*, 2006). However, to date, only few studies are reported about the efflux pumps contributing to FQs resistance in clinical *S. suis* (Escudero *et al.*, 2011). Therefore our study carried out and revealed that ABC transporter *SatA* and *SatB*, which are homologous to *PatA* and *PatB* of *Streptococcus pneumoniae*, can contribute to ciprofloxacin resistance in lab-derived *S. suis* mutants.

## MATERIALS AND METHODS

**Bacterial strains and drugs:** Four clinical FQs sensitive *S. suis* strains, JS12, JS13, JS14 and JS18, isolated from diseased animals from clinics as previously described by Carsenti-Etesse *et al.* (1999) and all the strains were induced by subinhibitory concentrations of ciprofloxacin or enrofloxacin. Nine FQs mutants were obtained and named as JS12E, JS12B, JS13E, JS13B, JS14E, JS14B, JS18E1, JS18E2 and JS18B. Ciprofloxacin, enrofloxacin (Sigma-Aldrich) and reserpine (Aladdin, shanghai, China) were dissolved according to the manufacturers′ instructions and were stored at -70°C prior to use.

**MIC measurement:** MICs were detected by microdilution method as recommended by Clinical and Laboratory Standards Institute (CLSI, 2008). *Staphylococcus aureus* ATCC25923 was used as a quality control strain. The synergistic effect of reserpine (20μg/mL, an efflux pump inhibitor) and FQs were also determined. The breakpoints of ciprofloxacin and enrofloxacin were ≥4 mg/L.

**Growth kinetics and growth inhibition assays:** The growth kinetics of the isolates JS12, JS13, JS14 and JS18 and their corresponding resistant strains used in current study were determined by screening $OD_{600}$ every 1 h as previously described (Garvey *et al.*, 2011). A little modified protocol based on previously described by Beyer *et al*. (2000) was used to assay the growth inhibition by ciprofloxacin.

**Detection of mutations within the QRDR of *gyrA, gyrB, parC* and*parE*:** Four original strains and five artificial induced strains were tested for mutations in DNA gyrase and topoisomerase IV by PCR of a total volume of 25 μL using the primers listed in Table 1.

**PCR amplification of *satA, satB, smrA* and sequencing:** Chromosomal DNA from all strains extracted by kit following the manufacturer′s instruction and used as template to amplify *satA*, *satB*, *smrA* and upstream promoter regions of 4 parental strains and corresponding resistant strains by using primers shown in Table 1 and the obtained PCR products were sequenced at Invitrogen (Shanghai, China).

**Detection of mRNA expression of related efflux pump:** The strains JS12, JS13, JS14 and JS18 and their corresponding resistant strains were chosen to detect the mRNA expression of *satA*, *satB* and *smrA*. All the strains were grown overnight in THB supplemented with 5% sterile bovine serum at 37°C in a 5% $CO_2$ atmosphere. Then the cells were inoculated in THB and grown for up to 10 h at 37°C in 5% $CO_2$ with a concentration of 1/4 × MIC ciprofloxacin added or not. Bacteria in log phase were harvested by centrifugation (12 000 g, 4 for 5 min) and the precipitation resuspended in lysozyme (30 mg/mL) with intense vortex for 30s. Total RNA was extracted and purified. Reverse transcription was carried out with Transgen Easy Script kit. Primes designed for the quantitative amplification of 16S rRNA, *satA, satB* and *smrA* are shown in Table 1. Real-time PCR was performed in an ABI 7300 cycler in 25 μL reaction mixtures containing 12.5 μL of ToYoBo SYBR Green master mix (2×) and 2 μL of cDNA. The 16S rRNA was used as a housekeeping gene to normalize the levels of the transcripts. Values of mRNA abundance were expressed as the fold change relative to the average value of control group. All data were presented as mean±SD, and analyzed by one-way ANOVA using SPSS 16.0 for Windows. The significance level was set at $P<0.05$.

**Detection of ciprofloxacin concentration in *S. suis* strains:** The concentrations of ciprofloxacin in sensitive and resistant strains were detected using HPLC method. Samples were prepared according to a previously described method with slight modifications (Chapman and Georgopapadakou, 1988). The concentration of ciprofloxacin was determined by HPLC under the following conditions: a Kromasil C18 column (150 mm × 4.6 mm, 5 μm), a flow rate of 1.0 ml/min, and UV detection with wavelengths of 277 nm. Accumulation experiments were performed three separate occasions. All data were presented as mean±SD, and analyzed by one-way ANOVA using SPSS 16.0 for Windows. The significance level was set at $P<0.05$.

## RESULTS

**Antibiotic susceptibility:** MICs of enrofloxacin and ciprofloxacin in the presence and absence of reserpine against 4 parental sensitive strains and the corresponding lab-derived resistant strains were determined in this study (Table 2). The results strongly showed that ciprofloxacin MIC values against resistant isolates could be reduced by reserpine except JS14E, but the MICs of enrofloxacin were not affected by reserpine, which indicated that an efflux pump was involved in ciprofloxacin not enrofloxacin. However, reserpine could not reverse the

**Table 1:** Primers used to amplify genes

| primers | Sequence (5'- 3') | Length (bp) |
|---|---|---|
| *gyrA* | CGCCGTATTTTGTATGGGATG/GTTCCGTTAACCAGAAGGTT | 377 |
| *gyrB* | GAAGGAGTGTCCGAATATGG/CTGGTGAAGATGTGCGTGAA | 596 |
| *parC* | AAGGACGGCAACACTTTTGAC/AGTGGGTTCTTTTTCCGTATC | 311 |
| *parE* | TGTGGTGGACGGCATTGTG/CCTCTACTAGCGGTCGCATAT | 532 |
| *satA* | CCGAGAATAACACCGACT/CACAACTTTCAAGGGACG | 589 |
| *satB* | CTCCCTCCCTTTCTGTGT/GTCGGTGGCTTTACTTCC | 905 |
| *smrA* | ATGGCTGCTCAGCTTTCTTT/AACATCCCTTACTTTCAAAT | 1319 |
| 16SrRNA | AGTAGGGAATCTTCGGCAATG/TTCGGGTGTTACAAACTCTCG | 1079 |
| Qu-16SrRNA | GTGAAGAAGGTTTTCGGATCGT/ GTAGTTAGCCGTCCCTTTCTGGT | 108 |
| Qu- *satA* | AACGACCGCCACCATCT/ACGCTCCGCTATTTACC | 131 |
| Qu-*satB* | TCGGCTCCGTATCGTGT/TCGGTGGCTTTACTTCC | 231 |
| Qu-*smrA* | AAGCAGAATTTGAAGGTG/AAGGGCATTAACAGATACCG | 156 |

**Table 2:** The MICs of enrofloxacin and ciprofloxacin against *S.suis* isolates with and without reserpine (ig/mL)

| strain | enrofloxacin | Enrofloxacin +reserpine | ciprofloxacin | Ciprofloxacin +reserpine |
|---|---|---|---|---|
| 18 | 1 | 1 | 1 | 1 |
| 18E1 | 64 | 64 | 32 | 16 |
| 18E2 | 128 | 128 | 64 | 8 |
| 18B | 4 | 4 | 32 | 2 |
| 12 | 2 | 2 | 2 | 2 |
| 12E | 16 | 16 | 16 | 8 |
| 12B | 128 | 128 | 64 | 16 |
| 13 | 0.5 | 0.5 | 1 | 1 |
| 13E | 128 | 128 | 64 | 32 |
| 13B | 2 | 2 | 32 | 8 |
| 14 | 0.5 | 0.5 | 0.5 | 0.5 |
| 14B | 16 | 16 | 128 | 32 |
| 14E | 32 | 32 | 8 | 8 |

**Table 3:** Point mutations of QRDR of *gyr*A, *gyr*B, *par*C and *par*E in induced resistant mutants

| Strains | *gyrA* | *gyrB* | *parC* | *parE* |
|---|---|---|---|---|
| 12B | - | - | - | - |
| 12E | Ser81Arg | - | Ser79Phe | |
| 13B | - | - | - | - |
| 13E | Ser81Arg | - | Ser79Phe | - |
| 14B | - | Ser256Phe | | - |
| 14E | Ser81Arg | | Ser79Phe | |
| 18B | - | Asp315Asn | - | Pro278Ser |
| 18E1 | Ser81Arg | Ser285Leu<br>288-291deletion<br>Leu554-556Ser | Ser79Phe | - |
| 18E2 | Ser81Arg | | Ser79Phe | |

MIC of ciprofloxacin and enrofloxacin against susceptible *S. suis* strains, suggesting that the intrinsic expression levels of this pump in *S. suis* is really low.

**Not all lab-derived resistant strains with mutations in QRDR of *gyrA, gyrB, parC and pare*:** In order to detect the mechanisms of FQs resistant isolates, the QRDRs of *gyrA, gyrB, parC and parE* were sequenced first. In most resistant strains, point mutations were found Ser81Arg of gyrA, Asp315Asn of *gyrB*, Ser79Phe of *parC* which are typical mutations as reported. Interestingly, there were no any point mutations detected in resistant strains JS12B and JS13B (Table 3). Taking together with MIC results and growth inhibition assays, it indicates that efflux pump may play an important role in mediating ciprofloxacin resistance in these strains.

**Growth inhibition assays by ciprofloxacin combined with reserpine:** Based on the above results that reserpine could affect the MIC of ciprofloxacin indicating an efflux existed in ciprofloxacin resistant strains, growth inhibition assays by ciprofloxacin combined with reserpine were determined. As shown in Fig.1A, the adding of reserpine stepped down the grown rate of artificial induced bacteria in broth with a ciprofloxacin concentration of 1/4 ×MIC, whereas in their parent susceptible strains almost no differences were observed. Results of parallel assays (Fig.1B) further confirmed that, the growth of sensitive strains with ciprofloxacin had no relation with reserpine. However, the adding of reserpine had evident effects on the growth of resistant strains. It was revealed that the efflux pump had stronger function in strains resistance to ciprofloxacin.

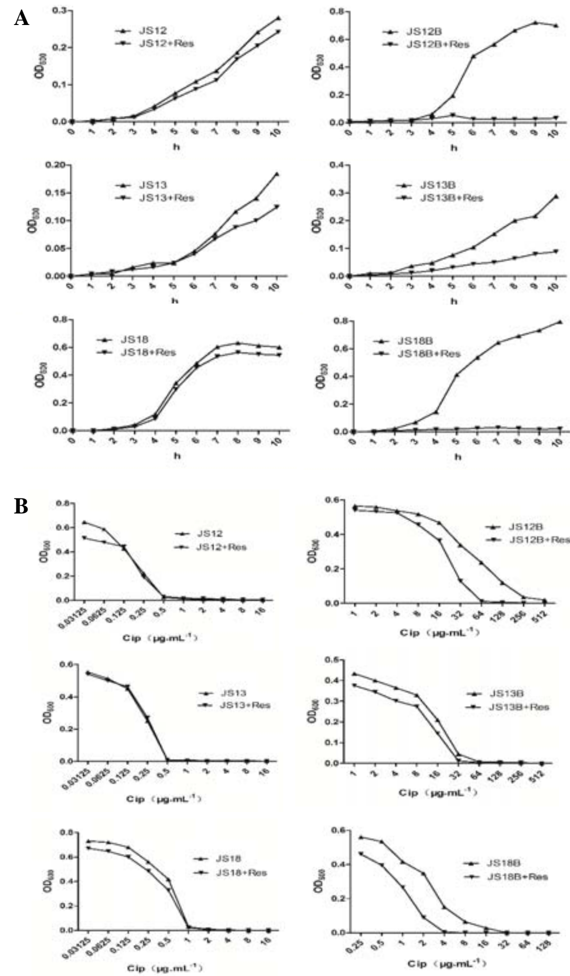


**Fig. 1:** Growth inhibition of *S. suis* by ciprofloxacin at one-fourth the MIC (Panel A) and $OD_{600}$ of *S. suis* at increasing ciprofloxacin concentrations (Panel B) in the presence or absence of reserpine

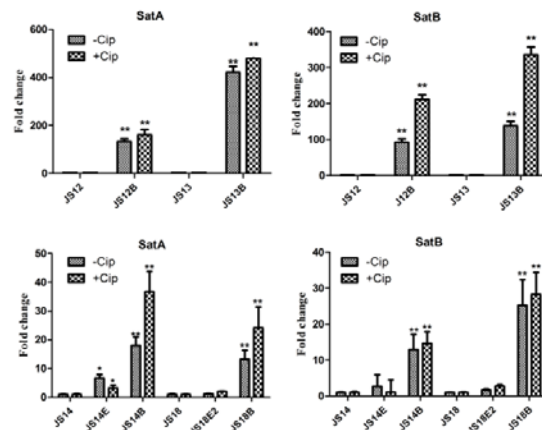


**Fig. 2:** Fold expression of *satA* and *satB* relative to that of parent susceptible strains respectively, measured by real time RT-PCR. In this study, resistant strains with (JS14E, JS14B, JS18B and JS18E2) and without (JS12B and JS13B) mutations within QRDR of *gyrA, gyrB, parC* and *parE* were selected. * P<0.05, ** P<0.01

**Overexpression of *SatA* and *satB* in the ciprofloxacin-resistant mutants:** Bioinformatics analysis revealed that SatA and SatB have close homologues and always occur

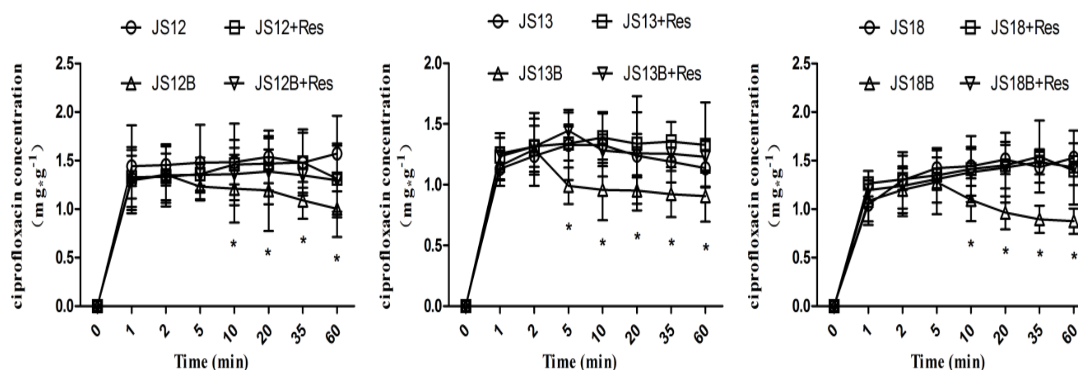


**Fig. 3:** Accumulation of ciprofloxacin (CIP) with or without reserpine (20 μg/ml) by representative isolates of *S. suis* with overexpression of *satA* and *satB*. *P<0.05

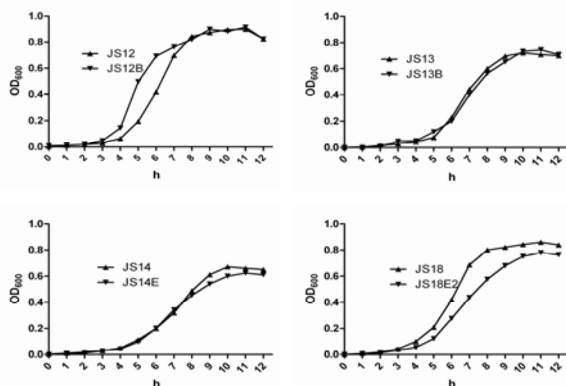


**Fig. 4:** The growth kinetics of a randomly chosen selection of isolates consisting of two isolates that overexpressed *satA* and *satB* (JS12B and JS13B)and two that did not(JS14E and JS18E2), were determined by measuring the optical density ($OD_{600}$) of cultures over time.

as an operon in *S. suis* and contributed to ciprofloxacin resistance. To determine whether over expression of *satA*, *satB* and *smrA* had a correction with ciprofloxacin resistance, the mRNA levels of *satA*, *satB* and *smrA* in resistant strains was compared with susceptible ones (Fig.2). Expression level of *satA* and *satB* were rising in all mutants regardless ciprofloxacin was added or not. Especially in strains JS12B and JS13B induced by ciprofloxacin without mutations in the QRDR, the mRNA levels of *satA* and *satB* were significantly higher than their parental susceptible strains (P<0.05). However, expression level of *smrA* was not found significant differences in any resistant isolates compared with their parental sensitive strains (Data not shown).

**Isolates overexpressing *satA* and *satB* accumulated significantly less ciprofloxacin:** To validate that overexpression of *satA* and *satB* has a correction with enhanced efflux function in the resistant isolate, the ciprofloxacin accumulation was measured in 3 resistant strains with higher expression level of *satA* and *satB*. The concentrations of ciprofloxacin in all tested resistant strains began to decrease after exposing to ciprofloxacin for5 to 10 min and significantly lower than those of parental sensitive strains (P<0.05). In the presence of reserpine (20 μg/ml), ciprofloxacin concentrations were significantly increased in resistant strains (Fig. 3). The results further confirmed that SatA and SatB played a role in decreasing ciprofloxacin accumulation.

**No mutations were found in *satA* or *satB* or upstream promoter regions:** To determine whether the decreased ciprofloxacin concentration was due to mutations of the transporter protein leading to functional change, the nucleotide sequences of the *satA*, *satB*, *smrA* and promoter region of four parental strains (JS12, JS13, JS14 and JS18) and corresponding resistant strains (JS12B, JS13B, JS14B and JS18B) were determined. The nucleotide sequences of those genes in JS12B, JS13B, JS14B and JS18B were identical to those of their corresponding parent sensitive strains. The results implied that the overexpression of *satA* and *satB* led to ciprofloxacin resistance, not due to occurring mutations in *satA*, *satB* and their putative promoter regions.

**The growth curve of parental sensitive strains and lab-derived resistant strains:** In order to estimate whether the overexpression of *satA* and *satB* in resistant strains may affect their fitness, the growth kinetics of strains with overexpressed *satA* and *satB* as well as those without overexpression were compared. The results (Fig. 4) showed that there was no significant alteration in growth rates between the two groups (P>0.05). From the result presented in this study, it could be indicated that overproducing *satA* and *satB* could not confer fitness costs on the resistant strains.

## DISCUSSION

In recent years, drug resistant pathogens are the hottest issue worldwide. One of the main reasons for the development and spread of resistance pathogen in environment is the misuse and overuse of antimicrobials in veterinary and human clinics (Khan *et al*., 2013). The resistance mechanism for various antimicrobial is well known, but the mechanism involved for the FQs resistance in *S. suis* is seldom reported. No doubt the FQs are extensively used in clinics. A number of studies have shown that there is no obvious difference in growth characteristics between sensitive and resistant strains. Hence, it was very important to find out that which mechanisms are involved for the development of FQs

resistance in *S. suis*. This study exposed two most important and frequent mechanisms responsible for the development of FQs resistance in *S. suis* which were also reported to be typical mutations conferring high resistance to FQs in other organisms (de la Campa *et al.*, 2003; Kawamura *et al.*, 2003; Escudero *et al.*, 2007; Lupien *et al.*, 2013). Asp315Asn mutation of *gyrB* and Pro278Ser alteration of *parE* are similar with those of previously found in *S. pneumonia* (Jones *et al.*, 2000). However, mutations of Ser256Phe, Leu554-556Ser and 288-291 deletion of *gyrB* have not been reported anywhere. The results here implied that mutations in *gyrA* and *parC* could mediate FQs resistance. In addition, it is very clear that these mutations have accumulated through being repeated exposure to ciprofloxacin for a prolonged period, as it is impossible that such resistant isolates with mutations could have emerged after a single exposure. In current study, at least five to seven exposures to ciprofloxacin or enrofloxacin would be required to gain the laboratory-derived mutants.

Most interestingly, there are no mutations detected in some resistant strains, therefore, efflux pump is predicted to be the main reason for causing resistance to ciprofloxacin based on the results of reserpine reversing the ciprofloxacin resistance and inhibition growth assays with reserpine. The assay of higher mRNA expression level of *satA*, *satB* and *smrA* in ciprofloxacin resistant strains and ciprofloxacin accumulation assay further proved efflux pump SatA and SatB, not SmrA, are associated with ciprofloxacin resistance in *S. suis.* The results are identical with previous reported in clinical resistant strains of *S. suis* (Escudero *et al.*, 2011). Our results showed that *satA* and *satB* always have similar expression tendency in the same strain simultaneous, suggesting that SatA and SatB need to interact together to make a functional drug efflux transporter, and they may work only as heterodimers as previously reported (Boncoeur *et al.*, 2012). One more interesting finding here was that ciprofloxacin but not enrofloxacin was affected by SatAB. The main reason for the difference is due to an increased bulkiness of the methylated substituent at position C-7 of enrofloxacin which could block enrofloxacin crossing from the efflux pump (Escudero *et al.*, 2011). It is also relevant from a therapeutic point that the selection of mutants with efflux overexpression or gy*rA*/*parC* mutations were determined by the FQs used in clinic. In current study, no obvious changes in growth rate were detected in mutants with or without overexpression of *satA* and *satB*. It is suggested that the overexpression of SatA and SatB may carry a low fitness cost to the lab-derived mutants and therefore the resistant isolates may be easily selected under a clinical setting.

**Conclusion:** It was revealed that overexpression of *satA* and *satB* adds in the occurrence of ciprofloxacin-resistance in *S. suis* isolates and overexpressing *satA* and *satB* isolates accumulate significantly less ciprofloxacin. Results of this study represent that SatA and SatB, not SmrA play a clinically relevant role in ciprofloxacin resistance. Further studies should be focused on characterizing SatAB and find out other possible transporters involved in FQs resistance in *S. suis* using mutagenic technology and proteomic methods.

**Acknowledgment:** The study was partially supported by the Natural Science Foundation of Jiangsu Province of China (No. BK2012771), the Fundamental Research Funds for the Central Universities (KYZ201105), Qing Lan Project and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

## REFERENCES

Beyer R, E Pestova, JJ Millichap, V Stosor, GA Noskin and LR Peterson, 2000. A convenient assay for estimating the possible involvement of efflux of fluoroquinolones by *Streptococcus pneumoniae* and *Staphylococcus aureus*: evidence for diminished moxifloxacin, sparfloxacin, and trovafloxacin efflux. Antimicrob Agents Chemother, 44: 798-801.

Boncoeur E, C Durmort, B Bernay, C Ebel, AM Di Guilmi, J Croize, T Vernet and JM Jault, 2012. PatA and PatB form a functional heterodimeric ABC multidrug efflux transporter responsible for the resistance of *Streptococcus pneumoniae* to fluoroquinolones. Biochem, 51: 7755-7765.

Carsenti-Etesse H, PM Roger, B Dunais, S Durgeat, G Mancini, M Bensoussan and P Dellamonica, 1999. Gradient plate method to induce *Streptococcus pyogenes* resistance. J Antimicrob Chemother, 44: 439-443.

Chapman JS and NH Georgopapadakou, 1988. Routes of quinolone permeation in *Escherichia coli*. Antimicrob Agents Chemother, 32: 438-442.

Clinical and Laboratory Standards Institute (CLSI), 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; 3rd Ed, CLSI document M31-A3 (ISBN 1-56238-659-X). Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

de la Campa AG, MJ Ferrandiz, F Tubau, R Pallares, F Manresa and J Linares, 2003. Genetic characterization of fluoroquinolone-resistant *Streptococcus pneumoniae* strains isolated during ciprofloxacin therapy from a patient with bronchiectasis. Antimicrob Agents Chemother, 47: 1419-1422.

Escudero JA, A San Millan, A Catalan, AG de la Campa, E Rivero, G Lopez, L Dominguez, MA Moreno and B Gonzalez-Zorn, 2007. First characterization of fluoroquinolone resistance in *Streptococcus suis*. Antimicrob Agents Chemother, 51: 777-782.

Escudero JA, A San Millan, B Gutierrez, L Hidalgo, RM La Ragione, M AbuOun, M Galimand, MJ Ferrandiz, L Dominguez, AG de la Campa and B Gonzalez-Zorn, 2011. Fluoroquinolone efflux in *Streptococcus suis* is mediated by SatAB and not by SmrA. Antimicrob Agents Chemother, 55: 5850-5860.

Feldman C and R Anderson, 2011. Bacteraemic pneumococcal pneumonia: current therapeutic options. Drugs, 71: 131-153.

Garvey MI, AJ Baylay, RL Wong and LJ Piddock, 2011. Overexpression of patA and patB, which encode ABC transporters, is associated with fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother, 55: 190-196.

Gottschalk M, J Xu, C Calzas and M Segura, 2010. *Streptococcus suis*: a new emerging or an old neglected zoonotic pathogen? Future Microbiol, 5: 371-391.

Higgins R and M Gottschalk, 2006. *Streptococcal diseases*. In Streptococcal diseases. 9th edn. Eds D'Allaire S, DJ Taylor and J Zimmerman, pp: 769-783. Blackwell Publishing, Ames.

Huang YT, LJ Teng, SW Ho and PR Hsueh, 2005. *Streptococcus suis* infection. J Microbiol Immunol Infect, 38: 306-313.

Hussain R, MT Javed, A Khan, F Mahmood and R Kausar, 2012. Mastitis and associated histo-pathological consequences in the context of udder morphology. Int J Agric Biol, 14: 947-952.

Khan A, R Hussain, MT Javed and F Mahmood, 2013. Molecular analysis of virulent genes (*coa* and *spa*) of *Staphylococcus aureus* involved in natural cases of bovine mastitis. Pak J Agric Sci, 50: 739-743.

Jones ME, DF Sahm, N Martin, S Scheuring, P Heisig, C Thornsberry, K Kohrer and FJ Schmitz, 2000. Prevalence of gyrA, gyrB, parC, and parE mutations in clinical isolates of *Streptococcus pneumoniae* with decreased susceptibilities to different fluoroquinolones and originating from Worldwide Surveillance Studies during the 1997-1998 respiratory season. Antimicrob Agents Chemother, 44: 462-466.

Kawamura Y, H Fujiwara, N Mishima, Y Tanaka, A Tanimoto, S Ikawa, Y Itoh and T Ezaki, 2003. First *Streptococcus agalactiae* isolates highly

resistant to quinolones, with point mutations in gyrA and parC. Antimicrob Agents Chemother, 47: 3605-3609.

Kerdsin A, S Dejsirilert, P Puangpatra, S Sripakdee, K Chumla, N Boonkerd, P Polwichai, S Tanimura, D Takeuchi, T Nakayama, S Nakamura, Y Akeda, M Gottschalk, P Sawanpanyalert and K Oishi, 2011. Genotypic profile of *Streptococcus suis* serotype 2 and clinical features of infection in humans, Thailand. Emerg Infect Dis, 17: 835-842.

Lupien A, DS Billal, F Fani, H Soualhine, GG Zhanel, P Leprohon and M Ouellette, 2013. Genomic characterization of ciprofloxacin resistance in a laboratory-derived mutant and a clinical isolate of *Streptococcus pneumoniae*. Antimicrob Agents Chemother, 57: 4911-4919.

Luzzaro F, A Endimiani, JD Docquier, C Mugnaioli, M Bonsignori, G Amicosante, GM Rossolini and A Toniolo, 2004. Prevalence and characterization of metallo-beta-lactamases in clinical isolates of pseudomonas aeruginosa. Diagn Microbiol Infect Dis, 48: 131-135.

Marrer E, K Schad, AT Satoh, MG Page, MM Johnson and LJ Piddock, 2006. Involvement of the putative ATP-dependent efflux proteins PatA and PatB in fluoroquinolone resistance of a multidrug-resistant mutant of *Streptococcus pneumoniae.* Antimicrob Agents Chemother, 50: 685-693.

Robertson GT, TB Doyle and AS Lynch, 2005. Use of an efflux-deficient *streptococcus pneumoniae* strain panel to identify ABC-class multidrug transporters involved in intrinsic resistance to antimicrobial agents. Antimicrob Agents Chemother, 49: 4781-4783.

Suankratay C, P Intalapaporn, P Nunthapisud, K Arunyingmongkol and H Wilde, 2004. *Streptococcus suis* meningitis in Thailand. Southeast Asian J Trop Med Public Health, 35: 868-876.

Tang J, C Wang, Y Feng, W Yang, H Song, Z Chen, H Yu, X Pan, X Zhou, H Wang, B Wu, H Zhao, Y Lin, J Yue, Z Wu, X He, F Gao, AH Khan, J Wang, GP Zhao, Y Wang, X Wang and GF Gao, 2006. Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. PLoS Med, 3: e151.

Yu H, H Jing, Z Chen, H Zheng, X Zhu, H Wang, S Wang, L Liu, R Zu, L Luo, N Xiang, H Liu, X Liu, Y Shu, SS Lee, SK Chuang, Y Wang, J Xu and W Yang, 2006. Human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis, 12: 914-920.