**NOTE** *Bacteriology*

# Contribution of Enhanced Efflux to Reduced Susceptibility of *Salmonella enterica* Serovar Choleraesuis to Fluoroquinolone and Other Antimicrobials

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ABSTRACT. We examined antimicrobial susceptibility and efflux systems in laboratory-derived mutants of *Salmonella enterica* serovar Choleraesuis selected by culture on fluoroquinolone-containing plates. The mutants exhibited decreased susceptibilities to quinolones and several other antimicrobials. Mutations in the *gyrA* gene were not always found in the mutants. Accumulation assays revealed that intracellular enrofloxacin concentrations were significantly lower in the mutants compared with parent isolates. Increased expression of *acrB* mRNA can explain the decreased susceptibilities to several antimicrobials but not in the case of carbonyl cyanide m-chlorophenylhydrazone (CCCP). Decreased susceptibility to CCCP may result from the increased expression of *emrA* mRNA. These results suggest that the enhancement of multiple efflux pumps is responsible for decreased susceptibilities to several antimicrobials in the laboratory-derived mutants.

KEY WORDS: antimicrobial resistance, efflux pump, fluoroquinolone resistance, laboratory-derived mutant, *S.* Choleraesuis.

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The emergence of fluoroquinolone-resistant *Salmonella enterica* serovar Choleraesuis strains is a major public health concern [7]. Fluoroquinolone resistance in *Salmonella* is caused by multiple mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV, decreased membrane permeability, increased activities of efflux pumps and plasmid-mediated quinolone resistance [3, 5, 11, 12]. Among these causes, mutations in the QRDR are the major mechanisms responsible for resistance to fluoroquinolones in *Salmonella* [2, 5, 7, 10, 12].

Several researchers have reported that enhancement of efflux pumps contributed to the emergence of fluoroquinolone resistance in *Salmonella* [23, 24]. The enhancement of efflux pumps as well as decreased membrane permeability has been observed in laboratory-derived mutants of *Salmonella* [3, 4, 6, 11]. To date, many kinds of efflux pumps have been identified in Gram-negative bacteria [16].

In this study, we selected mutant strains by passage on enrofloxacin-containing plates and examined their susceptibility to several antimicrobials as well as efflux systems in *S.* Choleraesuis.

Seven fluoroquinolone-susceptible strains of *S.* Choleraesuis were isolated from diseased pigs between 2001 and 2002 [1], and subjected to selection for quinolone-resistant mutants (Table 1). Each of the seven clinical isolates was plated on Mueller-Hinton (MH) agar plates containing 0.5 mg/*l* enrofloxacin. Any colonies that grew on these plates were subcultured. Based on minimum inhibitory concentrations (MICs) of nalidixic acid and enrofloxacin [14], mutants with decreased susceptibilities to nalidixic acid (MICs 16 and 32 mg/*l*) and enrofloxacin (MICs 0.25 and 0.5 mg/*l*) were designated as R1 strains. Strains resistant to nalidixic acid (MICs 64–1,024 mg/*l*) and enrofloxacin (MICs 0.5 and 1.0 mg/*l*) were selected and designated as R2 (Table 1). As an example, the mutants derived from strain 03-197-1 were designated as 03-197-1R1 and 03-197-1R2 depending on the MICs of nalidixic acid and enrofloxacin. All the isolates and mutants were stored at –80°C until used.

Mutations in the QRDRs of the *gyrA* gene were determined by direct DNA sequencing. Amplification of the gene and purification of the amplicon was performed as described by Giraud *et al.* [8]. Nucleotide sequences were determined using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA, U.S.A.) with a 3130 Genetic Analyzer (Applied Biosystems).

Of the seven resistant mutants, six mutants contained nucleotide changes in the *gyrA* gene accompanied by an amino acid substitution in the DNA gyrase A (GyrA) protein. Another mutant (582R2) contained nucleotide changes resulting in two amino acid substitutions (Table 2). Nucleotide sequences in the mutants with decreased susceptibilities were not accompanied with any amino acid substitutions in GyrA. It has been previously shown that the amino acid substitutions in GyrA contribute to resistance in quinolones [2, 5, 7, 10, 12].

The MICs of ampicillin, ceftiofur, chloramphenicol, and carbonyl cyanide m-chlorophenylhydrazone (CCCP) were determined by the micro-broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The MICs of oxytetracycline, erythromycin, trimethoprim, acriflavine and kanamycin were determined by the CLSI agar dilution method [14]. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Ten laboratory-derived mutants (03-197-1R1, 03-197-1R2, 03-228-1R1, 03-228-1R2, 916R1, 916R2, 143R1,

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Table 1. Susceptibilities of *S.* Choleraesuis parent isolates and laboratory-derived mutants to several antimicrobials

| Strain No.[a)] | Strain type | MIC (mg/*l*)[b)] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NAL | ERFX | ABPC | CTF | OTC | CP | ERY | TMP | ACF | KM | CCCP ($\mu$M) |
| 03-197-1 | Parent susceptible isolate | 4 | 0.0625 | >128 | 1 | 256 | 4 | 64 | 0.25 | 512 | 2 | 50 |
| 03-197-1R1 | Reduced susceptible mutant | **16** | **0.25** | >128 | **2** | **512** | **16** | **256** | **1** | **>512** | 2 | **100** |
| 03-197-1R2 | Resistant mutant | **64** | **1** | >128 | **2** | **512** | **16** | **256** | **1** | **>512** | 2 | **100** |
| 03-228-1 | Parent susceptible isolate | 2 | 0.0625 | <1 | 1 | 256 | 4 | 64 | 0.25 | 512 | 8 | 50 |
| 03-228-1R1 | Reduced susceptible mutant | **16** | **0.25** | **4** | **2** | **512** | **8** | **128** | **0.5** | **>512** | 8 | **100** |
| 03-228-1R2 | Resistant mutant | **512** | **0.5** | **4** | **2** | **512** | **8** | **128** | **0.5** | **>512** | 8 | **100** |
| 916 | Parent susceptible isolate | 4 | 0.0625 | 2 | 1 | 2 | 4 | 64 | 0.25 | 512 | 2 | 50 |
| 916R1 | Reduced susceptible mutant | **16** | **0.5** | **4** | **4** | **4** | **8** | **128** | **0.5** | **>512** | 2 | **200** |
| 916R2 | Resistant mutant | **64** | **0.5** | **8** | **4** | **16** | **32** | **256** | **1** | **>512** | 2 | **200** |
| 143 | Parent susceptible isolate | 4 | 0.0625 | <1 | 1 | 2 | 4 | 64 | 0.25 | 512 | 2 | 50 |
| 143R1 | Reduced susceptible mutant | **16** | **0.25** | **4** | **2** | **4** | **8** | **128** | **0.5** | **>512** | 2 | **100** |
| 143R2 | Resistant mutant | **128** | **0.5** | **2** | **2** | 2 | 4 | 64 | 0.25 | 512 | 2 | **100** |
| 1002 | Parent susceptible isolate | 4 | 0.0625 | 2 | 1 | 2 | 4 | 64 | 0.25 | 512 | 2 | 50 |
| 1002R1 | Reduced susceptible mutant | **16** | **0.25** | **4** | **2** | **4** | **16** | **256** | **0.5** | **>512** | 2 | **200** |
| 1002R2 | Resistant mutant | **512** | **1** | 2 | **2** | 2 | 4 | 64 | 0.25 | 512 | 2 | **100** |
| 582 | Parent susceptible isolate | 4 | 0.0625 | <1 | 1 | 2 | 4 | 64 | 0.25 | 512 | 2 | 50 |
| 582R1 | Reduced susceptible mutant | **16** | **0.25** | **4** | **2** | **4** | **8** | **128** | **0.5** | **>512** | 2 | **100** |
| 582R2 | Resistant mutant | **512** | **0.5** | **2** | 1 | 2 | 4 | 64 | 0.25 | 512 | 2 | **100** |
| ZSC-8 | Parent susceptible isolate | 8 | 0.125 | <1 | 1 | 256 | 128 | 64 | 0.25 | >512 | >512 | 100 |
| ZSC-8R1 | Reduced susceptible mutant | **32** | **0.25** | **4** | **2** | **512** | 128 | **128** | **0.5** | >512 | >512 | 100 |
| ZSC-8R2 | Resistant mutant | **1,024** | **1** | <1 | 1 | 256 | 128 | 64 | 0.25 | >512 | >512 | 100 |

MIC, minimum inhibitory concentration; NAL, nalidixic acid; ERFX, enrofloxacin; ABPC, ampicillin; CTF, ceftiofur; OTC, oxytetracycline; CP, chloramphenicol; ERY, erythromycin; TMP, trimethoprim; ACF, acriflavine; KM, kanamycin; CCCP, carbonyl cyanide m-chlorophenylhydrazone. a) R1 and R2 are laboratory-derived mutants selected from each susceptible parent isolate. R1 and R2 indicate the reduced-susceptiblility mutants (reduced susceptibility to NAL and ERFX) and resistant mutants (high level resistance to NAL and ERFX), respectively. b) Bold type indicates higher MICs compared with the respective parent isolates.

1002R1, 582R1 and ZSC-8R1) exhibited reduced susceptibilities to $\beta$-lactams (ampicillin and ceftiofur), oxytetracycline, chloramphenicol, erythromycin, trimethoprim and acriflavine when compared with each parent isolate (Table 1). Three laboratory-derived mutants (143R2, 1002R2 and 582R2) exhibited reduced susceptibilities to ampicillin and/or ceftiofur compared with each parent isolate, whereas their susceptibilities to oxytetracycline, chloramphenicol, erythromycin, trimethoprim and acriflavine were not altered. Susceptibility to CCCP was decreased in laboratory-derived mutants except in the case of two mutants (ZSC-8R1, ZSC-8R2) compared with each parent isolate. The susceptibility to kanamycin of all laboratory-derived mutants was equal to those of each parent isolate. Thus, the laboratory-derived mutants exhibited decreased susceptibilities to not only quinolones but also several other antimicrobials.

The amounts of intracellular enrofloxacin were measured by a fluorometric uptake assay according to the method of Piddock *et al.* [18] as described previously [24]. To investigate the effects of the efflux pump inhibitor on the concentration of intracellular enrofloxacin, the experiment was also carried out in the presence of CCCP (final concentration 100 $\mu$M) [21]. Data represents the mean value of the results from three independent experiments. Student's *t*-test was used to compare the results between and within experiments.

In the accumulation assays, the amounts of intracellular enrofloxacin in the mutants with decreased susceptibilities and those in the resistant mutants ranged from 6.1–8.7 *n*g/$10^5$ colony forming units (CFU) and 6.4–7.9 *n*g/$10^5$ CFU, respectively, which were significantly ($P<0.01$) lower than those obtained in the parent isolates (7.3–11.4 *n*g/$10^5$ CFU; Table 2). In the presence of CCCP, there was no significant difference in the intracellular enrofloxacin accumulation among parent isolates and mutants.

The present study demonstrated that decreased susceptibilities to quinolones in laboratory-derived mutants were associated with enhancement of efflux pumps. The enhancement of efflux pumps was commonly observed in the laboratory-derived mutants with and without amino acid substitutions in the GyrA. Giraud *et al.* suggested that enhancement of AcrAB is the primary mechanism before the mutation of QRDRs under fluoroquinolone selective pressure in *S.* Typhimurium [9]. Ricci and Piddock demonstrated that antimicrobials, including fluoroquinolone, could select for mutants of *Salmonella* with enhanced efflux mechanisms [22], and Chen *et al.* reported similar results. They suggested that the enhanced efflux mechanisms contributed to the development of resistance to fluoroquinolones and other antimicrobials in laboratory mutants of *S.* Typhimurium. Our results support their finding that enhancement of efflux in mutants selected by culture on fluoroquinolone-containing plates resulted in decreased sus-

ceptibility to quinolones and other antimicrobials.

To evaluate the genes conferring efflux pumps, quantitative PCR was applied to the detection and quantification of mRNAs. The preparations of cDNA were performed as described by Zheng *et al.* [25] with slight modifications. In brief, extraction of total RNAs from 5 m*l* of bacterial suspensions were carried out with an ISOGEN (Nippongene, Tokyo, Japan). The cDNA was synthesized from the RNA samples using the Primescript RT reagent kit (TaKaRa, Shiga, Japan). Quantitative PCR was performed with SYBR premix EX taq II (TaKaRa) on a One Step real-time system (Applied Biosystems) according to the manufacturer's instructions. The oligonucleotide primers used for the detection of cDNA specific for 16S rRNA were 5'-CCA GCA GCC GCG GTA AT-3' (forward) and 5'-TTT ACG CCC AGT AAT TCC GAT T-3' (reverse) resulting in a 57 bp amplicon. The *fsr* gene was amplified using the primers 5'-TCC GGC GGA CGT CAT GGT CT-3' (forward) and 5'-CCA GCA GCG GGC CCA ATG AA-3' (reverse), giving a 79 bp amplicon. All primers were designed with Primer3 (http://frodo.wi.mit.edu/primer3/). Primers specific for *acrB* (148 bp) [25], *emrA* (61 bp) [17] and *mdtK* (64 bp) [17] were used according to a previous report. The yield of amplicons from the *acrB*, *emrA, fsr* and *mdtK* genes were normalized to those originating from 16S rRNA. Within bacterial cells, the level of 16S rRNA was assumed to be transcribed at a constant rate throughout the growth conditions in this study. Data represent the mean value of results from three independent total RNA extractions. The correlation coefficient was calculated for the ratio of enrofloxacin accumulation in each parent isolate and the relative expression level of *acrB* mRNA in each parent isolate.

The expression of *acrB* mRNA was greater in all laboratory-derived mutants, except for the mutant ZSC-8R2, when compared with parent isolates (Table 2). The *acrB* mRNA expression level of the mutant ZSC-8R2 was unchanged from that of the parent isolate. The ratio of enrofloxacin accumulation in each parent isolate was significantly correlated with the relative expression of *acrB* mRNA (correlation coefficient: –0.64, $P<0.01$; data not shown). These results suggest that *acrB* mRNA expression may contribute to fluoroquinolone resistance in laboratory-derived mutants.

Several investigations have shown that the increased expression of *acrA* and *acrB* mRNAs resulted in decreased susceptibilities to quinolones, $\beta$-lactams, oxytetracycline, chloramphenicol, erythromycin, trimethoprim and acriflavine in Gram-negative bacteria, including *Salmonella* [3, 4, 13, 19, 20]. There is a possibility that the enhancement of other efflux pumps, like MdfA and MdtABC, makes additional contributions to the abovementioned antimicrobial susceptibilities [16]. However, enhancement of AcrAB, MdfA and MdtABC can explain the decreased susceptibility to the abovementioned antimicrobials, but not in the case of CCCP in laboratory-derived mutants.

Nishino and Yamaguchi showed that susceptibility to CCCP was decreased by the enhancement of EmrAB, Fsr [16] and MdtK, also known as YdhE, [15] efflux pumps. The expression of *emrA* mRNA was greater in laboratory-

Table 2. Characterization of *S.* Choleraesuis susceptible parent isolates and laboratory-derived mutants used in this study

| Strain No. | ERFX accumulation (*n*g/$10^5$ CFU) | | Mutations | n-Fold change in efflux pumps gene expression[a)] | | | |
|---|---|---|---|---|---|---|---|
| | Without CCCP | With CCCP | GyrA | *acrB* | *emrA* | *fsr* | *mdtK* |
| 03-197-1 | 9.9 | 58.7 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| 03-197-1R1 | 8.5 | 55.8 | Wild type | 4.9 ± 1.1 | 2.1 ± 0.3 | 1.4 ± 0.5 | 1.1 ± 0.2 |
| 03-197-1R2 | 7.4 | 51.0 | G81C | 5.3 ± 0.8 | 1.8 ± 0.4 | 0.9 ± 0.3 | 1.0 ± 0.3 |
| 03-228-1 | 9.8 | 56.0 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| 03-228-1R1 | 8.7 | 54.2 | Wild type | 1.6 ± 0.2 | 1.8 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.3 |
| 03-228-1R2 | 7.9 | 55.0 | G81C | 4.8 ± 0.3 | 1.6 ± 0.1 | 1.0 ± 0.3 | 1.4 ± 0.5 |
| 916 | 10.6 | 54.5 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| 916R1 | 8.3 | 53.8 | Wild type | 7.0 ± 1.7 | 1.6 ± 0.3 | 1.1 ± 0.2 | 0.8 ± 0.2 |
| 916R2 | 6.4 | 51.6 | L98V | 14.5 ± 2.2 | 1.7 ± 0.2 | 1.0 ± 0.2 | 1.4 ± 0.5 |
| 143 | 8.1 | 49.4 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| 143R1 | 6.1 | 48.3 | Wild type | 3.1 ± 0.5 | 1.8 ± 0.3 | 0.9 ± 0.3 | 0.6 ± 0.3 |
| 143R2 | 7.5 | 44.4 | D82N | 3.9 ± 0.8 | 1.8 ± 0.5 | 1.3 ± 0.4 | 1.1 ± 0.2 |
| 1002 | 11.4 | 55.2 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| 1002R1 | 7.4 | 52.9 | Wild type | 6.7 ± 1.1 | 2.6 ± 0.5 | 1.1 ± 0.2 | 1.1 ± 0.3 |
| 1002R2 | 7.8 | 55.0 | G81C | 1.4 ± 0.1 | 1.8 ± 0.4 | 1.0 ± 0.1 | 1.2 ± 0.3 |
| 582 | 7.3 | 52.3 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| 582R1 | 6.8 | 50.9 | Wild type | 4.3 ± 0.5 | 1.6 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.4 |
| 582R2 | 7.0 | 52.3 | D87G, L98V | 2.2 ± 0.6 | 1.4 ± 0.2 | 1.0 ± 0.1 | 0.9 ± 0.3 |
| ZSC-8 | 7.6 | 48.9 | Wild type | 1.0 | 1.0 | 1.0 | 1.0 |
| ZSC-8R1 | 7.2 | 47.8 | Wild type | 2.8 ± 0.2 | 1.1 ± 0.2 | 0.9 ± 0.2 | 1.0 ± 0.1 |
| ZSC-8R2 | 7.6 | 50.9 | D87N | 1.0 ± 0.1 | 0.9 ± 0.2 | 1.0 ± 0.2 | 1.0 ± 0.1 |

ERFX, enrofloxacin; CFU, colony forming units; CCCP, carbonyl cyanide m-chlorophenylhydrazone; GyrA, DNA gyrase A subunit.
a) Relative expression level of *acrB, emrA, fsr* and *mdtK* mRNAs compared to each parent isolate.

derived mutants, except for ZSC-8R1 and ZSC-8R2, compared with the parent isolate (Table 2). The mRNA expression of *fsr* and *mdtK* was unchanged in the laboratory-derived mutants (Table 2). These results suggest that decreased susceptibility to CCCP is caused by increased activities of EmrAB in laboratory-derived mutants. However, expression of EmrAB was not responsible for fluoroquinolone susceptibility in bacteria [16]. Further study is required to clarify the mechanism of enhancement of EmrAB in mutants selected with fluoroquinolone.

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REFERENCES

1. Asai, T., Namimatsu, T., Osumi, T., Kojima, A., Harada, K., Aoki, H., Sameshima, T. and Takahashi, T. 2010. Molecular typing and antimicrobial resistance of *Salmonella enterica* subspecies *enterica* serovar Choleraesuis isolates from diseased pigs in Japan. *Comp. Immunol. Microbiol. Infect. Dis.* **33**: 109–119.
2. Baucheron, S., Chaslus-Dancla, E. and Cloeckaert, A. 2004. Role of TolC and parC mutation in high-level fluoroquinolone resistance in *Salmonella enterica* serotype Typhimurium DT204. *J. Antimicrob. Chemother.* **53**: 657–659.
3. Baucheron, S., Tyler, S., Boyd, D., Mulvey, M. R., Chaslus-Dancla, E. and Cloeckaert, A. 2004. AcrAB-TolC directs efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium DT104. *Antimicrob. Agents Chemother.* **48**: 3729–3735.
4. Chen, S., Cui, S., McDermott, P. F., Zhao, S., White, D. G., Paulsen, I. and Meng, J. 2007. Contribution of target gene mutations and efflux to decreased susceptibility of *Salmonella enterica* serovar Typhimurium to fluoroquinolones and other anmicrobials. *Antimicrob. Agents Chemother.* **51**: 535–542.
5. Chiu, C. H., Su, L. H. and Chu, C. 2004. *Salmonella enterica* serotype Choleraesuis: epidemiology, pathogenesis, clinical disease, and treatment. *Clin. Microbiol. Rev.* **17**: 311–322.
6. Coldham, N. G., Randall, L. P., Piddock, L. J. and Woodward, M. J. 2006. Effect of fluoroquinolone exposure on the proteome of *Salmonella enterica* serovar Typhimurium. *J. Antimicrob. Chemother.* **58**: 1145–1153.
7. Esaki, H., Chiu, C. H., Kojima, A., Ishihara, K., Asai, T., Tamura, Y. and Takahashi, T. 2004. Comparison of fluoroquinolone resistance genes of *Salmonella enterica* serovar Choleraesuis isolates in Japan and Taiwan. *Jpn. J. Infect. Dis.* **57**: 287–288.
8. Giraud, E., Brisabois, A., Martel, J. L. and Chaslus-Dancla, E. 1999. Comparative studies of mutations in animal isolates and experimental in vitro- and in vivo-selected mutants of *Salmonella* spp. suggest a counterselection of highly fluoroquinolone-resistant strains in the field. *Antimicrob. Agents Chemother.* **43**: 2131–2137.
9. Giraud, E., Cloeckaert, A., Kerboeuf, D. and Chaslus-Dancla, E. 2000. Evidence for active efflux as the primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar Typhimurium. *Antimicrob. Agents Chemother.* **44**: 1223–1228.
10. Hansen, H. and Heisig, P. 2003. Topoisomerase IV mutations in quinolone-resistant salmonellae selected *in vitro*. *Microb. Drug Resist.* **9**: 25–32.
11. Hirai, K., Aoyama, H., Irikura, T., Iyobe, S. and Mitsuhashi, S. 1986. Differences in susceptibility to quinolones of outer membrane mutants of *Salmonella* typhimurium and *Escherichia coli*. *J. Antimicrob. Chemother.* **29**: 353–358.
12. Hopkins, K. L., Davies, R. H. and Threlfall, E. J. 2005. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int. J. Antimicrob. Agents* **25**: 358–373.
13. Li, X. Z. and Nikaido, H. 2004. Efflux-mediated drug resistance in bacteria. *Drugs* **64**: 159–204.
14. National Committee for Clinical Laboratory Standards. 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Second Edition: Approved Standard M31-A2., National Committee for Clinical Laboratory Standards, Wayne.
15. Nishino, K. 2003. [Whole genome approach of drug exporter genes and their regulatory networks] *Nippon Saikingaku Zasshi* **58**: 581–594 (in Japanese).
16. Nishino, K. and Yamaguchi, A. 2001. Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. *J. Bacteriol.* **183**: 5803–5812.
17. Nishino, K., Nikaido, E. and Yamaguchi, A. 2007. Regulation of multidrug efflux systems involved in multidrug and metal resistance of *Salmonella enterica* serovar Typhimurium. *J. Bacteriol.* **189**: 9066–9075.
18. Piddock, L. J., Jin, Y. F., Ricci, V. and Asuquo, A. E. 1999. Quinolone accumulation by *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. *J. Antimicrob. Chemother.* **43**: 61–70.
19. Piddock, L. J., White, D. G., Gensberg, K., Pumbwe, L. and Griggs, D. J. 2000. Evidence for an efflux pump mediating multiple antibiotic resistance in *Salmonella enterica* serovar Typhimurium. *Antimicrob. Agents Chemother.* **44**: 3118–3121.
20. Poole, K. 2001. Multidrug resistance in Gram-negative bacteria. *Curr. Opin. Microbiol.* **4**: 500–508.
21. Ricci, V. and Piddock, L. J. 2003. Accumulation of garenoxacin by *Bacteroides fragilis* compared with that of five fluoroquinolones. *J. Antimicrob. Chemother.* **52**: 605–609.
22. Ricci, V. and Piddock, L. J. 2009. Only for substrate antibiotics are a functional AcrAB-TolC efflux pump and RamA required to select multidrug-resistant *Salmonella* Typhimurium. *J. Antimicrob. Chemother.* **64**: 654–657.
23. Ricci, V., Tzakas, P., Buckley, A. and Piddock, L. J. 2006. Ciprofloxacin-resistant *Salmonella enterica* serovar Typhimurium strains are difficult to select in the absence of AcrB and TolC. *Antimicrob. Agents Chemother.* **50**: 38–42.
24. Usui, M., Uchiyama, M., Iwanaka, M., Nagai, H., Yamamoto, Y. and Asai, T. 2009. Intracellular concentrations of enrofloxacin in quinolone-resistant *Salmonella enterica* subspecies *enterica* serovar Choleraesuis. *Int. J. Antimicrob. Agents* **34**: 592–595.
25. Zheng, J., Cui, S. and Meng, J. 2009. Effect of transcriptional activators RamA and SoxS on expression of multidrug efflux pumps AcrAB and AcrEF in fluoroquinolone-resistant *Salmonella* Typhimurium. *J. Antimicrob. Chemother.* **63**: 95–102.