Resistance phenotypes and genotypes among multiple-antimicrobial-resistant Salmonella enterica subspecies enterica serovar Choleraesuis strains isolated between 2008 and 2012 from slaughter pigs in Okinawa Prefecture, Japan

Masanao MATAYOSHI1*, Takashi KITANO2, Tetsu SASAKI1 and Masaji NAKAMURA2)

1) Okinawa Prefecture Central Livestock Hygiene Service Center, 2505 Ozato, Ozato, Nanjyo, Okinawa 901–1202, Japan
2) Okinawa Prefecture Central Meat Inspection Center, 2015 Ozato, Ozato, Nanjyo, Okinawa 901–1202, Japan

(Received 22 December 2014/Accepted 20 January 2015/Published online in J-STAGE 21 February 2015)

ABSTRACT. A total of 349 Salmonella enterica subspecies enterica serovar Choleraesuis (S. Choleraesuis) strains, which were isolated between 2008 and 2012 from 349 pigs at two slaughterhouses in Okinawa Prefecture, Japan, were investigated for antimicrobial susceptibility and the presence of antimicrobial resistance genes. All isolates were resistant to at least four antimicrobial agents. The antimicrobial agents for which isolates showed a high incidence of resistance were as follows: ampicillin (100%) and streptomycin (100%), followed by gentamicin (99.7%), oxytetracycline (97.5%), sulfamethoxazole/trimethoprim (99.4%), nalidixic acid (40.1%) and oxolinic acid (40.1%). All isolates were sensitive to cefotaxime, ceftriaxone, colistin, fosfomycin, enrofloxacin, ofloxacin and danofloxacin. The predominant resistance phenotypes and genotypes were: resistance to ampicillin, streptomycin, gentamicin, oxytetracycline and sulfamethoxazole/trimethoprim (58.5%, 204/349) and blaTEM-strA-strB-aadA1-aadA2-ascC2-ise (B)-sul1-sul2-dfrXII-dfrXIII (36.1%, 126/349). The quinolone resistance-determining regions (QRDRs) of gyrA, gyrB, parC and parE of the quinolone-resistant isolates (n=12) showed amino acid substitutions of Ser-83→Phe or Asp-87→Tyr in GyrA and Ser-107→Ala in ParC. To our knowledge, this is the first report on the molecular characterization of antimicrobial resistance among S. Choleraesuis strains in Japan.

KEYWORDS: antimicrobial resistance, pig, resistance genes, Salmonella Choleraesuis, zoonosis

doi: 10.1292/jvms.14-0683; J. Vet. Med. Sci. 77(6): 705–710, 2015

The emergence of antimicrobial-resistant Salmonella enterica has become a major public health hazard worldwide. In Southeast Asia, pigs may be an important reservoir of Salmonella for humans, and there have been many reports on isolating antimicrobial-resistant S. enterica from pork, pork products and the slaughter environment [2–5, 8, 9, 28, 29]. Salmonella enterica subspecies enterica serovar Choleraesuis (S. Choleraesuis) is a highly swine-adapted organism that causes several different disease syndromes [6, 15]. This pathogen is a zoonotic agent that is transmitted to humans via contaminated food (e.g., raw meat and liver), usually causing septicemic infections that require antimicrobial treatment [6, 7]. S. Choleraesuis infection is designated as a notifiable infectious disease in Japan. Thus, diseased pigs containing the organism should be condemned at postmortem inspection. In recent years, multidrug-resistant (MDR) S. Choleraesuis has become a public concern in Japan’s neighbors, that is, Taiwan and other countries [5, 19, 20, 25–28].

In Okinawa Prefecture, S. Choleraesuis was not detected at slaughterhouses until May 2008. Since then, S. Choleraesuis isolates have been regularly recovered from slaughter pigs (85–163 cases per year between 2008 and 2012). In general, pigs can remain subclinical carriers of Salmonella, and these pigs are found in meat inspections, contributing to economic losses of swine producers. Therefore, antimicrobial therapy is essential in the treatment of S. Choleraesuis infection [6, 15]. Consequently, collection of detailed information on the in vitro activities of antimicrobial agents is an important strategy for both meat producers and clinicians.

The objectives of this study were 1) to investigate the antimicrobial resistance phenotypes and genotypes of S. Choleraesuis isolates from slaughter pigs and 2) to provide trace-back information on this foodborne zoonosis.

Salmonella Choleraesuis isolates: A total of 349 strains of Salmonella Choleraesuis were isolated from organs, livers, spleens, mesenteric lymph nodes and tracheal lymph nodes, of 349 slaughter pigs from 25 farms in 8 municipalities at slaughterhouses in Okinawa Prefecture, Japan, between May 2008 and January 2012 (Table 2). The isolates were identified biochemically (API® 20 E, bioMérieux, Marcy-l’Étoile, France) and were serotyped by slide and tube agglutination tests with commercially available antisera (Denka Seiken Co., Ltd., Tokyo, Japan).

Antimicrobial susceptibility test: Antimicrobial susceptibility testing was carried out using the disk agar diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [10]. Mueller-Hinton agar (Nippon Becton Dickinson, Fukushima, Japan) batches were used as the culture medium. The following antimicrobial agents were used: ampicillin (ABPC, 10 μg), streptomycin (SM, 10 μg), kanamycin (KM, 30 μg), gentamicin (GM,
Table 1. PCR primers used for antimicrobial resistance genes and sequencing of QRDR

| Antimicrobial family | Resistance gene | Forward PCR primer sequence (5’-3’) | Reverse PCR primer sequence (5’-3’) | Reference |
|----------------------|-----------------|------------------------------------|------------------------------------|-----------|
| **Beta-lactams**     |                 |                                    |                                    |           |
| blaTEM               | GAGTCTTCCTCTCAATTTTCC | ACCAATCTTACTTACTTACTTTG | [22] |
| blaSHV               | TCGCCGTTGTAATGCTTCTC | CGGAGATATTCACCAACTAAATGG | [22] |
| blaOXA               | TCAACTTCTCTTCTTTCGAA | GAGTCGTTTAAATGGGTGTA | [1] |
| blaPSE               | GCAAGATGGGCCAGCATCACTC | GAGCTACGTTAATGTGTTTCCA | [8] |
| blaCMY               | GACGCCCTCTTCTTCACCA | TGGAACAAAGGCTACGTA | [29] |
| **Aminoglycosides**  |                 |                                    |                                    |           |
| strA                 | TGGCCAGGGAACCAAGGAGG | AGGTCCGATGCAAGCCTTC | [21] |
| strB                 | GGGGCTACCTTCTTCGCCA | GTGTTGTTTAGAATGGTGA | [1] |
| aadA1                | TATCAGAGGAGTGGGTTCTCAT | GTTCCATGCGTTAAGGTTTCCA | [24] |
| aadA2                | TGGTGTGTTGCTGCTTCA | AGTTGACCCAGCTTGCTG | [22] |
| aadB                 | GAGCCGAAATCTGGCTCCTTG | CTTCGGGTCTTTCACCAAGC | [22] |
| ahpA1                | ATGGGCTGCCGTAATGCTC | CTCACCGGAGCCGTTCC | [22] |
| ahpA2                | AGAACAGTGTAGTGGCAGCTCA | GCTTCTGACGAAATACGACG | [22] |
| **Tetracycline**     |                 |                                    |                                    |           |
| tet (A)              | GTGGAACCAAACATACCC | GAAAGCAGGAGCAGTAGAT | [22] |
| tet (B)              | CTTATACGCGACATGCTTGC | ACTCCGTTTCTTTCGCC | [22] |
| tet (C)              | ACTTGGAGGCACATACGAC | CTCAAACTCCGACCAACC | [22] |
| tet (D)              | TGGGCGATGCTGACAGAAG | CAGCACCCGTGATTTTTTCC | [22] |
| tet (E)              | TATAATGGCAACAGGAC | TTCATACCCATCCATTCCA | [22] |
| tet (G)              | GGGTCTTATGGGTTCTCTA | CCAAGAAACGGAACCGAGTC | [24] |
| **Phenicols**        |                 |                                    |                                    |           |
| floR                 | CGGCCATCTTCCACATCCC | GATCAGGCGCAGCTGTC | [22] |
| cmI                  | CGCCGCGCTGGTTCTTATC | CACCTGCGTCGCCATCATTAG | [18] |
| catA1                | AGTGTGCTAATGCTCACTTAA | TGTTATCCTACGAATCTGCC | [22] |
| **Sulfonamides/ trimethoprim** | | | | |
| sulI                 | TTTGCATCTGAACTCACC | ATGCAAACAACTCCTGTC | [22] |
| sulII                | CGCGCATGCTCAACAATACG | GTTGCCGAGATGCTC | [22] |
| dhfrI                | AAAAATGGGATTGTTGGAAGG | GGGTAAAAACTGGCCTAAAAATTG | [22] |
| dhfrII               | CTGCAAAAGCCGAAAACGG | AGCAAGTTAATGGTGGTGAAG | [22] |
| dhfrIV               | GTGAATGCCCCTGATCCTCAC | TTTGGATTTGGTCCAC | [22] |
| dhfrIX               | TCTAAATCAGATTTGCGTCGTC | TTGTTTCTAGAAATGGTGCG | [22] |
| dhfrX                | ACCAGACGATCTGCAATCTG | TTGATGACATACGCAATGAC | [4] |
| dhfrXII               | AAATCTCCGGTGACGAGAG | CCGTTGACGGAGATTTGGAAG | [4] |
| dhfrXIII              | CAGCTTGACGAGAATTCTT | CACAAAACTGTTGGATGACAC | [22] |
| **QRDR**             |                 |                                    |                                    |           |
| gyrA                 | GCTGAAGAGCTCCTACTCGG | GGTGCCTGATACGCGTACC | [9] |
| gyrB                 | GGGGCTGCCGATTTAGGCC | TGTAGACGCTGCGTCC | [9] |
| parC                 | GTACGTCATGATGATCTGTG | TTTCTCAGGTTGGCGCTCG | [9] |
| parE                 | GGGATCGCGAATATCACCAG | CAGTTGTCAGTACGCGGC | [9] |

10 µg), cefoxime (CMX, 30 µg), ceftiofur (CTF, 30 µg) oxytetracycline (OTC, 30 µg), chloramphenicol (CP, 30 µg), sulfamethoxazole/trimethoprim (ST, 23.75/1.25 µg), fosfomycin (FOM, 50 µg), nalidixic acid (NA, 30 µg), oxolinic acid (OA, 10 µg), enrofloxacin (ERFX, 5 µg), orbifloxacin (OBFX, 10 µg) and danofloxacin (DNFX, 5 µg). Antibiotic discs used in the test were purchased from Nippon Becton Dickinson, except for oxolinic acid, enrofloxacin, orbifloxacin and danofloxacin, which were kindly donated by Eiken Chemical Co., Ltd. (Tokyo, Japan), Bayer Yakuhin, Ltd. (Tokyo, Japan), Merial Japan Ltd. (Tokyo, Japan) and Pfizer Japan Inc. (Tokyo, Japan), respectively. Reading of inhibition zones was interpreted according to the manufacturer’s instructions. Quality control strains were routinely used: Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853.

Detection of antimicrobial resistance genes: The choice of resistance genes to be studied was based on their relative importance, as observed in resistant Salmonella enterica isolates. For PCR amplification of antimicrobial resistance genes, 32 different oligonucleotide primer sets were used in the study (Table 1). We tested genotypes for resistance to ampicillin (blaTEM, blaSHV, blaPSE, blaOXA and blaCMY), streptomycin (strA, strB, aadA1 and aadA2), kanamycin (aadB, aphA1 and aphA2), gentamicin [aadB, aac (3) IV, aacC2 and aphA2], oxytetracycline [tet (A), tet (B), tet (C), tet (D), tet (E) and tet (G)], chloramphenicol (floR, cmI and catA1), and sulfamethoxazole/trimethoprim [sulI, sulII (sulfonamide resistance), dhfrI, dhfrV, dhfrVII, dhfrIX, dhfrX, dhfrXII and dhfrXIII (trimethoprim resistance)]. All primers were commercially synthesized (Hokkaido System Science Co., Ltd., Sapporo, Japan). To prepare DNA templates, bacterial cells were suspended in distilled water and boiled for 10 min, and the cells were pelleted by centrifugation for 1 min. Amplifications were carried out with 5 µl of the supernatants. PCR was performed in a final volume of 25
Salmonella Choleraesuis isolates: None of the isolates showed H₂S production, which was determined by Api 20 E systems, and therefore, they were classified into biotype Choleraesuis (H₂S−).

Antimicrobial susceptibility test: The isolates showed high levels of resistance to ampicillin (100% of the isolates were resistant) and streptomycin (100%), followed by gentamicin (99.7%), oxytetracycline (99.7%), sulfamethoxazole/trimethoprim (99.4%), nalidixic acid (40.1%) and oxolinic acid (40.1%). In contrast, a single isolate was resistant to kanamycin and chloramphenicol. None of the isolates exhibited resistance to cefuroxime, cefotiofur, colistin, fosfomycin, enrofloxacin, orbifloxacin or danofloxacin.

All isolates could be grouped into five phenotypes (Table 3). The most frequent multiple resistance phenotype was ABPC-SM-GM-OTC-ST (58.5%) followed by ABPC-SM-GM-OTC-ST-NA/OA (40.4%). All isolates were resistant to four or more antimicrobials.

Detection of antimicrobial resistance genes: Antimicrobial-resistant genotypes are shown in Table 3.

Beta-lactams. All 349 beta-lactam-resistant isolates possessed blaTEM genes. None of the isolates tested were positive for blaSHV, blaPSE, blaOXA and blaCMY.

Aminoglycosides. Of the nine aminoglycoside resistance genes for which tests were conducted, 99.4% of the streptomycin-resistant isolates possessed strA, strB and aadA1, while 98.8% possessed strA, strB and aadA2. One kanamycin-resistant isolate possessed only the aphA1 gene. One gentamicin-resistance gene, aacC2, was found among the gentamicin-resistant isolates, and one isolate was negative for the genes tested.

Tetracycline. Of the six tetracycline-resistance genes targeted, tet (A) and tet (B) were detected. The tet (B) gene was found exclusively in 99.4% of all tetracycline-resistant isolates, except for one isolate possessing tet (A), and only one tetracycline-resistant isolate possessed none of the tet genes tested.

Phenicols. One chloramphenicol-resistant isolate possessed only the catA1 gene.

Sulfamethoxazole/trimethoprim. Among the sulfamethoxazole/trimethoprim-resistant isolates, almost all (98.3 to 100%) of the resistant isolates possessed sul1 and/or sul2 genes, and both the dhfrIIId and dhfrXIII genes were detected in all sulfamethoxazole/trimethoprim-resistant isolates.

Sequencing of gyrA, gyrb, parC and parE genes: Twelve isolates harbored mutations that encoded an amino acid substitution within the QRDRs of gyrA and parC. Nine isolates possessed a change at Ser-83 to Phe (TCC→TTC), and three
isolate... resistance to streptomycin and gentamicin is... is a serious concern. Furthermore, conventional antimicrobials, such as ampicillin, sulfamethoxazole/trimethoprim, fosfomycin, extended-spectrum cephalosporins and fluoroquinolones, have been recommended for invasive salmonellosis. Thus, resistance to streptomycin and gentamicin is a serious concern. Furthermore, conventional antimicrobials, such as ampicillin, sulfamethoxazole/trimethoprim, fosfomycin, extended-spectrum cephalosporins and fluoroquinolones, have been recommended for invasive salmonellosis. Hence, the high prevalence of resistance to ampicillin and sulfamethoxazole/trimethoprim may cause serious problems with respect to public health as a result of zoonotic infections.

A single enrofloxacin-resistant S. Choleraesuis isolate was recovered from a diseased pig in the Japanese Veterinary Antimicrobial Resistance Monitoring Program [3, 13, 14]. Therefore, fluoroquinolone-resistant S. Choleraesuis is not widespread in Japan. Our current data are consistent with these findings.

In this study, aacC2, which has rarely been detected among Salmonella [4, 21], was exclusively detected from gentamicin-resistant isolates. Furthermore, trimethoprim-resistant isolates exclusively possessed both the *dhfrXII* and *dhfrXIII* genes, and almost all isolates displayed very similar genotype-phenotype correlations in antimicrobial resistance. Thus, all isolates showed strong similarities at both phenotypic and genotypic levels, suggesting that the isolates were of clonal origin. The hypothesis may be supported by the finding of indistinguishable pulsed-field gel electrophoresis (PFGE) patterns for twenty S. Choleraesuis strains, isolated between May 2008 and November 2008 at a slaughterhouse in Okinawa Prefecture, (Goto N, personal communication, 2009). However, further expandable studies involving PFGE analysis and other typing methods are necessary to more precisely determine whether or not the multiple-antimicrobial-resistant S. Choleraesuis isolates are derived from the same origin and are widely distributed.

Interestingly, the prevalence of nalidixic acid and oxolinic acid-resistant isolates has significantly increased, from 0% in 2008 to 87.5% in 2012 (2009, 15.4%; 2010, 58.1%; 2011, 79.1%). Nalidixic acid has not been approved for therapeutic use in pigs in Japan, whereas oxolinic acid was first approved for food-producing animals including cattle, swine and poultry in 1975. Oxolinic acid has been particularly used for decades to treat colibacillosis and salmonellosis in piglets with diarrhea. In general, inappropriate or intensive use of antimicrobials in farming practices can potentially lead to the emergence of antimicrobial resistance among bacteria. However, oxolinic acid had rarely been used on the farms investigated. The reason for the rapid dissemination of S. Choleraesuis resistant to an old quinolone is unknown.

As in other bacteria, the mechanisms of resistance to quinolone in Salmonella have been attributed to point mutations in the QRDRs of gyrA and parE. In this study, all isolates exhibited multidrug resistance to four or more of the antimicrobials tested. Almost all (98.9%) displayed very similar antimicrobial resistance phenotypes, such as ABPC-SM-GM-OTC-ST (58.5%) and ABPC-SM-GM-OTC-ST-NA/OA (40.4%). In the Japanese veterinary fields, especially the swine industry, ampicillin, ABPC-SM-GM-OTC-ST (58.5%) and ABPC-SM-GM-OTC-ST-NA/OA (40.4%). In the Japanese veterinary fields, especially the swine industry, ampicillin, ABPC-SM-GM-OTC-ST-NA/OA (40.4%). In the Japanese veterinary fields, especially the swine industry, ampicillin, sulfamethoxazole/trimethoprim, nalidixic acid, oxolinic acid.

### Table 3. Antimicrobial resistance phenotypes and genotypes of Salmonella Choleraesuis from slaughter pigs

| Resistance phenotype | No. of isolates (%) | Resistance genotype |
|----------------------|---------------------|---------------------|
| ABPC-SM-KM-OTC-CP-ST | 1 (0.3)             | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aphA1*, *tetA*, *catA1*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
| ABPC-SM-GM-OTC-ST-NA/OA | 74 (21.2)         | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 62 (17.8)          | *blaTEM*, *strA*, *strB*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 3 (0.9)            | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 1 (0.3)            | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 1 (0.3)            | *blaTEM*, *strA*, *strB*, *aadA2*, *aacC2*, *tetB*, *sul2*, *dhfrXII*, *dhfrXIII* |
| ABPC-SM-GM-OTC-STM     | 126 (36.1)         | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 73 (20.9)          | *blaTEM*, *strA*, *strB*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 1 (0.3)            | *blaTEM*, *strA*, *strB*, *aadA2*, *aacC2*, *tetB*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 2 (0.6)            | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 1 (0.3)            | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2*, *dhfrXII*, *dhfrXIII* |
|                        | 1 (0.3)            | *blaTEM*, *aadA2*, *tetB*, *sul1*, *dhfrXII*, *dhfrXIII* |
| ABPC-SM-GM-OTC         | 1 (0.3)            | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *tetB*, *sul1*, *sul2* |
|                        | 1 (0.3)            | *blaTEM*, *aadA2*, *aacC2*, *tetB* |
| ABPC-SM-GM-STM         | 1 (0.3)            | *blaTEM*, *strA*, *strB*, *aadA1*, *aadA2*, *aacC2*, *sul2*, *dhfrXII*, *dhfrXIII* |

**Total**: 349 (100)

ABPC, ampicillin; SM, streptomycin; KM, kanamycin; GM, gentamicin; OTC, oxytetracycline; CP, chloramphenicol; ST, sulfamethoxazole/trimethoprim; NA, nalidixic acid; OA, oxolinic acid.
revealed that similar amino acid substitutions were detected in the nalidixic and oxolinic acid-resistant isolates. Ling et al. [20] reported that mutations in gyrA conferred low-level fluoroquinolone resistance, while addition of another gyrA mutation together with parC and/or parE mutation increased the resistance to a high level. Furthermore, in Japan, an S. Choleraesuis isolate with a single mutation in gyrA showed minimum inhibitory concentrations of 512 µg/ml for nalidixic acid and 2 µg/ml for enrofloxacin, and those of S. Choleraesuis with double mutations in gyrA were 512 µg/ml and 4 µg/ml, respectively [13]. Our results were consistent with these findings.

In conclusion, fluoroquinolones were very active against S. Choleraesuis. Thus, these agents are recommended for treatment and control of this foodborne pathogen. However, in Okinawa, use of fluoroquinolones for therapy and prophylaxis has gradually increased on swine farms, and thus, excessive use of fluoroquinolones may cause the emergence of resistance to these drugs in the future. The widespread nature of MDR S. Choleraesuis carrying multiple resistance genes is of serious concern, and it should be carefully monitored.

REFERENCES

1. Ahmed, A. M., Furuta, K., Shimomura, K., Kasama, Y. and Shimamoto, T. 2006. Genetic characterization of multidrug resistance in Shigella spp. from Japan. J. Med. Microbiol. 55: 1685–1691. [Medline] [CrossRef]

2. Aarestrup, F. M., Lertworapreecha, M., Evans, M. C., Bangtrakanonth, A., Chalermchaikit, T., Hendriksen, R. S. and Wegener, H. C. 2003. Antimicrobial susceptibility and occurrence of resistance genes among Salmonella enterica serovar Weltevreden from different countries. J. Antimicrob. Chemother. 52: 715–718. [Medline] [CrossRef]

3. 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. [Medline] [CrossRef]

4. Chen, S., Zhao, S., White, D. G., Schroeder, C. M., Lu, R., Yang, H., McDermott, P. F., Ayers, S. and Meng, J. 2004. Characterization of multiple-antimicrobial-resistant salmonella serovars isolated from retail meats. Appl. Environ. Microbiol. 70: 1–7. [Medline] [CrossRef]

5. Chiu, C. H., Wu, T. L., Su, L. H., Chu, C., Chia, J. H., Kuo, A. J., Chien, M. S. and Lin, T. Y. 2002. The emergence in Taiwan of fluoroquinolone resistance in Salmonella enterica serotype choleraesuis. N. Engl. J. Med. 346: 413–419. [Medline] [CrossRef]

6. 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. [Medline] [CrossRef]

7. Chu, C., Chiu, C. H., Wu, W. Y., Chu, C. H., Liu, T. P. and Ou, J. T. 2001. Large drug resistance virulence plasmids of clinical isolates of Salmonella enterica serovar Choleraesuis. Antimicrob. Agents Chemother. 45: 2299–2303. [Medline] [CrossRef]

8. Chuanchuen, R., Pathanasophon, P., Khemtong, S., Wannaprast, W. and Padungtod, P. 2008. Susceptibilities to antimicrobials and disinfectants in Salmonella isolates obtained from poultry and swine in Thailand. J. Vet. Med. Sci. 70: 595–601. [Medline] [CrossRef]

9. Chuanchuen, R. and Padungtod, P. 2009. Antimicrobial resistance genes in Salmonella enterica isolates from poultry and swine in Thailand. J. Vet. Med. Sci. 71: 1349–1355. [Medline] [CrossRef]

10. Clinical and Laboratory Standards Institutes. 2014. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fourth Informational [Suppl]: M100–S24 Clinical and Laboratory Standards Institutes, Wayne.

11. Cloeckaert, A. and Chaslus-Daniel, E. 2001. Mechanisms of quinolone resistance in Salmonella. Vet. Res. 32: 291–300. [Medline] [CrossRef]

12. Eaves, D. J., Randall, L., Gray, D. T., Buckley, A., Woodward, M. J., White, A. P. and Piddock, L. J. 2004. Prevalence of mutations within the quinolone resistance-determining region of gyrA, gyrB, parC, and parE and association with antibiotic resistance in quinolone-resistant Salmonella enterica. Antimicrob. Agents Chemother. 48: 4012–4015. [Medline] [CrossRef]

13. 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. [Medline] [CrossRef]

14. Esaki, H., Moriya, A., Ishihara, K., Kojima, A., Shiroki, S., Tamura, Y. and Takahashi, T. 2004. Antimicrobial susceptibility of Salmonella isolated from cattle, swine and poultry (2001-2002): report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. J. Antimicrob. Chemother. 53: 266–270. [Medline] [CrossRef]

15. Fedorka-Cray, P. J., Gray, J. T. and Wray, C. 2003. Salmonella infections in pigs. pp. 191–207. In: Salmonella in Domestic Animals, 2nd ed. (Way, C. and Way, A. eds.), CABI Publishing, Oxford.

16. Griggs, D. J., Ginsberg, K. and Piddock, L. J. V. 1996. Mutations in gyrA gene of quinolone-resistant Salmonella serotypes isolated from humans and animals. Antimicrob. Agents Chemother. 40: 1009–1013. [Medline] [CrossRef]

17. Huang, T. M., Chang, Y. F. and Chang, C. F. 2004. Detection of mutations in the gyrA gene and class I integron from quinolone-resistant Salmonella enterica serovar Choleraesuis isolates in Taiwan. Vet. Microbiol. 100: 247–254. [Medline] [CrossRef]

18. Keyes, K., Hudson, C., Maurer, J. J., Thayer, S., White, D. G. and Lee, M. D. 2000. Detection of florfenicol resistance genes in Escherichia coli isolated from sick chickens. Antimicrob. Agents Chemother. 44: 421–424. [Medline] [CrossRef]

19. Kuo, H. C., Lauderdale, T. L., Lo, D. Y., Chen, C. L., Chen, P. C., Liang, S. Y., Kuo, J. C., Liao, Y. S., Liao, C. H., Tsao, C. S. and Chiou, C. S. 2014. An association of genotypes and antimicrobial susceptibility with serovar Choleraesuis isolates in Taiwan. Vet. Microbiol. 49: 3567–3573. [Medline] [CrossRef]

20. Lynne, A. M., Rhodes-Clark, B. S., Bliven, K., Zhao, S. and Foley, S. L. 2008. Antimicrobial resistance genes associated with Salmonella enterica serovar newport isolates from food animals. Antimicrob. Agents Chemother. 52: 353–356. [Medline] [CrossRef]

21. Maynard, C., Fairbrother, J. M., Bekal, S., Sanschagrin, F., Levesque, R. C., Brousseau, R., Masson, L., Lariviere, S. and Harel, J. 2003. Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Chemother. 47: 3214–3221.
23. Piddock, L. J. V., Ricci, V., McLaren, I. and Griggs, D. J. 1998. Role of mutation in the gyrA and parC genes of nalidixic-acid-resistant salmonella serotypes isolated from animals in the United Kingdom. J. Antimicrob. Chemother. 41: 635–641. [Medline] [CrossRef]

24. Randall, L. P., Cooles, S. W., Osborn, M. K., Piddock, L. J. and Woodward, M. J. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the U.K. J. Antimicrob. Chemother. 53: 208–216. [Medline] [CrossRef]

25. Sirichote, P., Bangtrakulnonth, A., Tianmanee, K., Unahalekhaka, A., Oulai, A., Chittaphithakchai, P., Kheowrod, W. and Hendriksen, R. S. 2010. Serotypes and antimicrobial resistance of Salmonella enterica ssp in central Thailand, 2001-2006. Southeast Asian J. Trop. Med. Public Health 41: 1405–1415. [Medline]

26. Su, L. H., Teng, W. S., Chen, C. L., Lee, H. Y., Li, H. C., Wu, T. L. and Chiu, C. H. 2011. Increasing ceftriaxone resistance in Salmonellae, Taiwan. Emerg. Infect. Dis. 17: 1086–1090. [Medline] [CrossRef]

27. Tamang, M. D., Nam, H. M., Kim, A., Lee, H. S., Kim, T. S., Kim, M. J., Jung, G. C., Jung, S. C. and Lim, S. K. 2011. Prevalence and mechanisms of quinolone resistance among selected nontyphoid Salmonella isolated from food animals and humans in Korea. Foodborne Pathog. Dis. 8: 1199–1206. [Medline] [CrossRef]

28. Yang, B., Qu, D., Zhang, X., Shen, J., Cui, S., Shi, Y., Xi, M., Sheng, M., Zhi, S. and Meng, J. 2010. Prevalence and characterization of Salmonella serovars in retail meats of marketplace in Shaanxi, China. Int. J. Food Microbiol. 141: 63–72. [Medline] [CrossRef]

29. Zhao, S., White, D. G., McDermott, P. F., Friedman, S., English, L., Ayers, S., Meng, J., Maurer, J. J., Holland, R. and Walker, R. D. 2001. Identification and expression of cephapycinase bla(CMY) genes in Escherichia coli and Salmonella isolates from food animals and ground meat. Antimicrob. Agents Chemother. 45: 3647–3650. [Medline] [CrossRef]