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Characterization of *Salmonella* Food Isolates with Concurrent Resistance to Ceftriaxone and Ciprofloxacin

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Abstract

Foodborne salmonellosis is an important public health problem worldwide. Most human *Salmonella* infections occur through the consumption of contaminated food of animal origin. The study reported the first isolation of two *Salmonella enterica* serovar Oranienburg strains from pork in China with concurrent resistance to ciprofloxacin and ceftriaxone. Both isolates also showed resistance to norfloxacin, trimethoprim-sulfamethoxazole, and chloramphenicol, and an elevated minimal inhibitory concentraton of azithromycin; one strain was also resistant to amikacin, gentamicin, tetracycline, and amoxicillin-clavulanic acid. *Salmonella* ceftriaxone resistance was due to the production of IncN plasmidborne CTX-M-14 ESBL, and their ciprofloxacin resistance was mediated by target mutations and efflux pump activity. This is the first time that ceftriaxone- and ciprofloxacin-resistant *Salmonella* was reported in meat products, which may be due to the uses of antibiotics in animal production. The study warrants the continuous surveillance of multidrug-resistant *Salmonella* in meat products and cautious use of antibiotics in food animals.

Introduction

Foodborne salmonellosis is an important public health problem worldwide and the leading cause of foodborne illnesses in many countries such as the United States and China (Gomez *et al.,* 1997). Most human *Salmonella* infections occur through the consumption of contaminated food of animal origin, such as poultry, beef, pork, eggs, and milk (Gomez *et al.,* 1997). Although antibiotics are not essential for the treatment of most cases of salmonellosis, they can be lifesaving in invasive infections, which normally occur in children and elderly people. Ceftriaxone and ciprofloxacin are the choices of treatment for invasive *Salmonella* infections in humans (Glynn *et al.,* 1998; Hohmann, 2001). Resistance of quinolone and fluoroquinolone are often associated with point mutations at the Quinolone Resistance Determining Region (QRDR) of *gyrA* and *parC* (Hooper, 2001). In *Salmonella*, it is demonstrated that quinolone resistance often results from single mutation in *gyrA*, whereas fluoroquinolone resistance is always due to double mutation in *gyrA* and single mutation in *parC* (Chen *et al.,* 2007). In addition to chromosomal mutation, plasmid-mediated quinolone resistance (PMQR), including derivatives of quinolone resistance proteins (Qnr), aminoglycoside acetyltransferase *aac(6′)-Ib-cr*, and quinolone efflux pump QepA, have also been described in quinolone- and fluoroquinolone-resistant *Salmonella* isolates (Nordmann and Poirel, 2005; Robicsek *et al.,* 2006). Resistance to extended-spectrum β-lactams are mainly due to intracellular production extended-spectrum β-lactamases (ESBLs). CTX-M- and OXA-type β-lactamases are commonly reported in *Enterobacteriaceae*, in which CTX-M-14 and OXA-30 are discovered frequently in *Salmonella* (Antunes *et al.,* 2004; Lewis *et al.,* 2007). The increasing trend of ceftriaxone- or ciprofloxacin-resistant *Salmonella* infections, in particular in Asia, poses a huge threat to human health due to the limited choices of treatment. In this study, we report the first isolation of multidrug-resistant *Salmonella enteric* serovar Oranienburg with concurrent resistance to both ceftriaxone and ciprofloxacin from meat products in China.

Methods

Salmonella *isolation*

Meat products, including pork and chicken, were purchased from Guangdong Province, China and Hong Kong SAR. These samples were subjected to *Salmonella* isolation using selective XLT4 agar plates containing 16 μg/mL of ceftriaxone and 2 μg/mL of ciprofloxacin as previously described (Andrews *et al.*, 2011). *Salmonella* isolates were confirmed by API20E (BioMerieux, Mercy, France) and

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polymerase chain reaction (PCR) assay targeting *invA* gene (Rahn *et al.,* 1992). Serotyping of *Salmonella* was done by multiplex PCR approach as previously described (Kim *et al.,* 2006).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on *Salmonella* isolates by agar dilution method and interpreted following Clinical and Laboratory Standards Institute (CLSI) instructions (CLSI, 2010). Fourteen antimicrobials were tested: nalidixic acid, ciprofloxacin, norfloxacin, ampicillin, amikacin, gentamicin, tetracycline, cefotaxime, ceftriaxone, ceftiofur, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, chloramphenicol, and meropenem. Azithromycin susceptibility was tested by broth microdilution method as previously described (Sjolund-Karlsson *et al.,* 2011). *Escherichia coli* American Type Culture Collection (ATCC) 25922 and 35218, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213 were used as quality controls. Effect of efflux pumps on fluoroquinolone and cephalosporin susceptibility was assessed by determining minimal inhibitory concentration (MIC) on ciprofloxacin, norfloxacin, and ceftriaxone by broth microdilution method following CLSI instructions with the presence of an efflux pump inhibitor, phenylalanine arginine β-naphthylamide (PAβN) of a final concentration of 30 μg/mL (CLSI, 2010).

β-Lactamases, PMQRs, and QRDR mutation determination

The presence of ESBLs was determined using PCR assay targeting most of the β-lactamases as previously described (Dallenne *et al.,* 2010). Full-length β-lactamase genes were amplified and sequenced by specific primers (Table 1). The QRDR of *gyrA*, *gyrB*, *parC,* and *parE* were amplified by PCR as previously described (Chen *et al.,* 2007), sequenced, and then compared to the genes from wild-type *Salmonella enterica* serovar Typhimurium LT2 to determine the target gene mutations. The presence of PMQR genes, *qnrA, qnrB, qnrC, qnrD, qnrS, qepA, oqxAB,* and *aac(6')Ib-cr* was determined by PCR using primers described previously (Chen *et al.,* 2007; Deng *et al.,* 2011b). Insertion sequences (ISs) were frequently detected upstream of ESBLs and responsible for the capture and mobilization of the antibiotic resistance genes. Forward primers targeting insertion sequences IS*CR1*, IS*Ecp1,* and IS*903,* and reverse primer $bla_{\text{CTX-M-14}}$-R (Table 1) were used to determine the linkage of insertion sequence with $bla_{\text{CTX-M-14}}$. All PCR products were sequenced to confirm the correct sequence of the products.

Plasmid replicon typing and conjugation experiment

Plasmids were isolated using Plasmid miniprep kit (Qiagen). Replicon typing of plasmids was performed by PCR-based method as previously described (Carattoli *et al.,* 2005). Conjugation experiment was performed in *Salmonella* isolates using a sodium azide-resistant *E. coli* J53 as recipient strain as previously described (Jacoby *et al.,* 2003). Transconjugants were selected on LB agar plates containing ceftriaxone (16 μg/mL) and sodium azide (100 μg/mL).

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed following the PulseNet PFGE protocol for *Salmonella* using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with pulse times of 2.16–63.8 s as previously described (Ribot *et al.,* 2006). Clonal relatedness were analyzed by Bionumerics with the use of Dice coefficient and a parameter of 0.5% for optimization and band matching tolerance.

Results and Discussion

From April to August 2011, 330 and 150 pork and chicken samples were purchased from different supermarkets and farmer's markets in Guangzhou, China and Hong Kong, SAR, respectively. Two *Salmonella* isolates were obtained from two pork samples from Guangzhou, and no positive *Salmonella* was isolated from meat samples from Hong Kong. The two *Salmonella* isolates were designated as *Salmonella* S166 and S284, and were isolated at different times and markets in Guangzhou city. S166 was isolated from pork that originated from farm A, and S284 was from pork that originated from farm B. The serotype of these two *Salmonella* isolates was determined to be *Salmonella enterica* serovar Oranienburg. PFGE characterization of these two isolates showed 60% similarity and thus belonged to different PFGE types (Table 2).

Both strains exhibited resistance to nine antimicrobials tested: ampicillin, cefotaxime, ceftriaxone, ceftiofur, nalidixic acid, ciprofloxacin, norfloxacin, trimethoprim-sulfamethoxazole, and chloramphenicol. *Salmonella* S166 also showed resistance to amikacin, gentamicin, amoxicillin-clavulanic acid, and tetracycline (Table 2).

Both *Salmonella* isolates were multidrug-resistant, and S166 showed only susceptibility to meropenem, a reserved antibiotic for severe clinical Gram-negative pathogen infections. Most significantly, both isolates showed concurrent resistance to both ceftriaxone and ciprofloxacin, which are current choices for treatment of human *Salmonella* clinical

Table 1. Primers and Polymerase Chain Reaction (PCR) Procedures Used in This Study

Primer	*Sequence (5'to 3')*	*Expected size*	*PCR conditions*
$bla_{CTX\text{-}M\text{-}14}$-*F*	TCGAATGGTGACAAAGAGAGTGCA	875	95°C for 3min, (95°C 30s, 55°C 30s,
$bla_{CTX\text{-}M\text{-}14}$-*R*	TACTTTACAGCCCTTCGGCGATGAT		72°C 1min)× 30, 72°C for 10min
$bla_{OXA\text{-}1}$-*F*	GACTTTATAAATTTAGTGTGTTTA	829	
$bla_{OXA\text{-}1}$-*R*	ACGTTATGAAAAACACAATACAT		
armA-F	CAATCAGGGGCAGTTATCA	529	
armA-R	CCCTATAACCTTCGAATC		
ISEcp1-F	CTGCAAACGGTGCTGCGGAA		95°C for 3min, (95°C 30s, 60°C 30s,
IS903-F	CGCAGCGTCAGTGAACCCCC		68°C 2.5min)× 30, 68°C for 10min
ISCR1-F	AGACGCCGTGGAAGCGTGTG		

TABLE 2. CHARACTERISTICS OF TWO *SALMONELLA* ORANIENBURG ISOLATED FROM FOOD

			QRDR mutations			Fluoroquinolones MICs (μg/mL)				Other antimicrobial MICs (μg/mL)										
Strain	PFGE patterns	No. of plasmid	GyrA	ParC	β-lactamases	NAL	CIP	CIP + PAβN	NOR	NOR + PAβN	AMP	AMK	GEN	TEL	CTO	CTX	CRO	CRO + PAβN	AMC	SXT	CHL	AZR
S166	A	6	S83F, D87N	S80R	CTX-M-14, OXA-1	>64	>16	2	>64	8	>64	>128	>64	>32	>64	>64	>64	>64	16/8	>8/156	>64	≥64
S284	B	1	S83F, D87N	S80R	CTX-M-14, OXA-1	>64	>16	2	>64	16	>64	<8	<4	<4	>64	>64	>64	>64	8/4	>8/156	>64	≥64

PFGE, pulsed-field gel electrophoresis; NAL, nalidixic acid; CIP, ciprofloxacin; NOR, norfloxacin; AMK, amikacin; AMP, ampicillin; GEN, gentamicin; TEL, tetracycline; CTO, ceftiofur; CTX, cefotaxime; CRO, ceftriaxone; AMC, amoxicillin-clavulanic acid; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; AZR, azithromycin; PAβN, phenylalanine arginine β-naphthylamide.

infections. *Salmonella* Choleraesuis that showed concurrent resistance to both ceftriaxone and ciprofloxacin has only been reported in a children's hospital in Taiwan, which was suggested to be due to the nosocomial uses of antibiotics (Chiu *et al.,* 2004; Su *et al.,* 2005). In this study, the isolation of multidrug-resistant *Salmonella* Oranienburg that showed concurrent resistance to both ceftriaxone and ciprofloxacin from meat products in China suggests a potential threat to human health. In addition, these two isolates showed a MIC of ≥64 μg/mL for azithromycin, which is increasingly being adopted for treating multidrug-resistant salmonellae infection. Currently, there is no established breakpoint of azithromycin for *Enterobacteriaceae*. A study investigating azithromycin susceptibility of clinical and environmental *Salmonella* isolates revealed that majority of *Salmonella* would have a MIC of 16 μg/mL (Sjolund-Karlsson *et al.,* 2011). The relatively high azithromycin MIC showed by two isolates in this study warrants further researches on *Salmonella* azithromycin susceptibility. Furthermore, most of *Salmonella* can cause clinical infections and *Salmonella* Oranienburg identified in this study has been reported to cause foodborne illness outbreaks in the United States, Europe, and Asia (CDC, 2007; Kumao *et al.,* 2002). *Salmonella* Oranienburg can cause invasive human infections and antibiotic treatment is necessary (Kumao *et al.,* 2002). Therefore, the selection of *Salmonella* Oranienburg and maybe other more common serotype such as *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis in the future with concurrent resistance to both ciprofloxacin and ceftriaxone, which may result from the uses of antibiotics in food animals, can cause human health consequence in the future.

The antibiotic usage was investigated in the two farms where isolates was obtained in this study. Fluoroquinolones, such as ciprofloxacin, norfloxacin, and levofloxain have been used in these farms since 1980s for treatment and growth promotion purposes. Ceftiofur, one of the veterinary use β-lactam, has been used as disease treatment purpose since 2002 due to its high costs. However, due to the increasing trend of multidrug resistant animal pathogens, the uses of these two drugs are becoming more frequent and with higher doses in recent years, which may trigger the emergence of *Salmonella* with concurrent resistance to both ciprofloxacin and ceftriaxone. The antibiotic uses in animal production in China will keep increasing and the development of these double drug-resistant *Salmonella* will be speeding up. The consequence may be seen in near future.

Conjugation experiments were conducted for these two isolates using *E. coli* J53 as recipient strain. However, no successful transconjugants could be obtained. Plasmids were isolated from these two *Salmonella* isolates and showed different profiles. S166 contained several plasmids with different sizes, whereas S284 contained only one plasmid of ~24kb that was also found in S166. This plasmid was purified from both strains through gel extraction and was determined to belong to IncN group. An ESBL gene, $bla_{\text{CTX-M-14}}$, and a non-ESBL gene, $bla_{\text{OXA-1}}$, were identified on this plasmid, whereas no β-lactamase gene has been detected from the chromosomal DNA. CTX-M-14 and OXA-1 are commonly found β-lactamases in *Enterobacteriaceae.* Although less frequent than CMY-2 β-lactamase in *Salmonella*, CTX-M-14 has been reported in ceftriaxone-resistant *Salmonella* in different parts of the world (Chiu *et al.,* 2004; Fey *et al.,* 2000; Jin and Ling, 2006;

Li *et al.,* 2005; Romero *et al.,* 2004; Tamang *et al.,* 2011). $bla_{CTX\text{-}M\text{-}14}$ carried on IncN plasmid was first reported in *Salmonella,* and $bla_{CTX\text{-}M\text{-}14}$ has never been reported in *Salmonella* isolates in China. In addition, an insertion sequence IS*903* was detected upstream of the $bla_{CTX\text{-}M\text{-}14}$ from both isolates. A recent study has shown the presence of $bla_{CTX\text{-}M\text{-}14}$ linking to IS*903* on a 95-kb IncI1 conjugative plasmid in a clinical isolate of *Salmonella* Enteritidis (Bado *et al.,* 2012). The first identification of $bla_{CTX\text{-}M\text{-}14}$ on a IncN plasmid in this study suggesting the possible transmission of $bla_{CTX\text{-}M\text{-}14}$ within plasmids through insertion sequence such as IS903. Interestingly, the IncN plasmid from S166 also carried *armA* gene, a 16S rRNA methylase gene, which is responsible for its resistance to aminoglycosides. This is also the first report of *armA* found on IncN plasmid in *Salmonella*. Further studies will be needed to determine the genetic structures of $bla_{CTX\text{-}M\text{-}14}$ and *armA* on IncN type of plasmid.

Double mutations on GyrA (S83F, D87N) and single mutation on ParC (S80R) were found on both strains. No GyrB mutation was found. The result is consistent with a previous study that showed only mutations on GyrA and ParC mediate quinolone and fluoroquinolone resistance (Chen *et al.,* 2007). Both isolates were negative to all PMQR genes. The contribution of *Salmonella* efflux pump to their multidrug-resistances was determined using efflux pump inhibitor, PAβN. The presence of PAβN significantly reduced the MIC of ciprofloxacin, while not norfloxacin or ceftriaxone suggesting the contribution of efflux pump to ciprofloxacin resistance in these two *Salmonella*. Further study is needed to understand the different mechanisms of norfloxacin and ciprofloxacin resistances in these *Salmonella* isolates.

Conclusion

The study reported the first isolation of ceftriaxone and ciprofloxacin-resistant *Salmonella* from meat product. *Salmonella* ceftriaxone resistance was due to the production of IncN plasmidborne CTX-M-14 ESBL and their ciprofloxacin resistance was mediated by target mutations and efflux pump activity. The emergence of ceftriaxone and ciprofloxacin-resistant *Salmonella* may be due to the uses of antibiotics in animal production. The study warrants the continuous surveillance of multidrug-resistant *Salmonella* in meat products and cautious use of antibiotics in food animals.

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Disclosure Statement

No competing financial interests exist.

References

Andrews WH, Jacobson A, Hammack T. Chapter 5: *Salmonella.* In: *Bacteriological Analytical Manual (BAM).* U.S. Food and Drug Administration, 2011. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm, accessed March 1, 2011.

Antunes P, Machado J, Sousa JC, Peixe L. Dissemination amongst humans and food products of animal origin of a *Salmonella* Typhimurium clone expressing an integron-borne OXA-30 beta-lactamase. J Antimicrob Chemother 2004;54:429–434.

Bado I, García-Fulgueiras V, Cordeiro NF, Betancor L, Caiata L, Seija V, Robino L, Algorta G, Chabalgoity JA, Ayala JA, Gutkind GO, Vignoli R. First human isolate of *Salmonella enterica* serotype Enteritidis harboring *blaCTX-M-14* in South America. Antimicrob Agents Chemother 2012;56:2132–2134.

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005;63:219–228.

[CDC] Centers for Disease Control and Prevention. *Salmonella* Oranienburg infections associated with fruit salad served in health-care facilities–northeastern United States and Canada, 2006. MMWR Morb Mortal Wkly Rep 2007;56:1025–1028.

Chen S, Cui S, McDermott PF, Zhao S, White DG, Paulsen I, Meng J. Contribution of target gene mutations and efflux to decreased susceptibility of *Salmonella enterica* serovar Typhimurium to fluoroquinolones and other antimicrobials. Antimicrob Agents Chemother 2007;51:535–542.

Chiu CH, Su LH, Chu C, Chia JH, Wu TL, Lin TY, Lee YS, Ou JT. Isolation of *Salmonella enterica* serotype Choleraesuis resistant to ceftriaxone and ciprofloxacin. Lancet 2004;363:1285–1286.

[CLSI] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI Document M100-S20*. Wayne, PA: CLSI, 2010.

Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 2010;65:490–495.

Deng Y, He L, Chen S, Zheng H, Zeng Z, Liu Y, Sun Y, Ma J, Chen Z, Liu JH. F33:A-:B- and F2:A-:B- plasmids mediate dissemination of rmtB-blaCTX-M-9 group genes and *rmtB-qepA* in *Enterobacteriaceae* isolates from pets in China. Antimicrob Agents Chemother 2011a;55:4926–4929.

Deng Y, Zeng Z, Chen S, He L, Liu Y, Wu C, Chen Z, Yao Q, Hou J, Yang T, Liu JH. Dissemination of IncFII plasmids carrying *rmtB* and *qepA* in *Escherichia coli* from pigs, farm workers and the environment. Clin Microbiol Infect 2011b;17:1740–1745.

Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, Bradford PA, Angulo FJ, Hinrichs SH. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. N Engl J Med 2000;342:1242–1249.

Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo F.J. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N Engl J Med 1998;338:1333–1338.

Gomez TM, Motarjemi Y, Miyagawa S, Kaferstein FK, Stohr K. Foodborne salmonellosis. World Health Stat Q 1997;50:81–89.

Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis 2001;32:263–269.

Hooper DC. Emerging mechanisms of fluoroquinolone resistance. Emerg Infect Dis 2001;7:337–341.

Jacoby GA, Chow N, Waites KB. Prevalence of plasmid-mediated quinolone resistance. Antimicrob Agents Chemother 2003;47:559–562.

Jin Y, Ling JM. CTX-M-producing *Salmonella* spp. in Hong Kong: An emerging problem. J Med Microbiol 2006;55:1245–1250.

Kim S, Frye JG, Hu J, Fedorka-Cray PJ, Gautom R, Boyle DS. Multiplex PCR-based method for identification of common clinical serotypes of *Salmonella enterica* subsp. enterica. J Clin Microbiol 2006;44:3608–3615.

Kumao T, Ba-Thein W, Hayashi H. Molecular subtyping methods for detection of *Salmonella enterica* serovar Oranienburg outbreaks. J Clin Microbiol 2002;40:2057–2061.

Lewis JS, 2nd, Herrera M, Wickes B, Patterson JE, Jorgensen JH. First report of the emergence of CTX-M-type extended-spectrum beta-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother 2007;51:4015–4021.

Li WC, Huang FY, Liu CP, Weng LC, Wang NY, Chiu NC, Chiang CS. Ceftriaxone resistance of nontyphoidal *Salmonella enterica* isolates in Northern Taiwan attributable to production of CTX-M-14 and CMY-2 beta-lactamases. J Clin Microbiol 2005;43:3237–3243.

Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. J Antimicrob Chemother 2005;56:463–469.

Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galán JE, Ginocchio C, Curtiss R 3rd, Gyles CL. Amplification of an invA gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. Mol Cell Probes 1992;6:271–279.

Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 2006;3:59–67.

Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis 2006;6:629–640.

Romero L, Lopez L, Martinez-Martinez L, Guerra B, Hernandez JR, Pascual A. Characterization of the first CTX-M-14-producing *Salmonella enterica* serotype Enteritidis isolate. J Antimicrob Chemother 2004;53:1113–1114.

Sjölund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, Fedorka-Cray P, Zhao S, Crump JA, Whichard JM. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* isolates from the United States. Antimicrob Agents Chemother 2011;55:3985–3989.

Su LH, Wu TL, Chia JH, Chu C, Kuo AJ, Chiu CH. Increasing ceftriaxone resistance in *Salmonella* isolates from a university hospital in Taiwan. J Antimicrob Chemother 2005;55:846–852.

Tamang MD, Nam HM, Kim TS, Jang GC, Jung SC, Lim SK. Emergence of extended-spectrum beta-lactamase (CTX-M-15 and CTX-M-14)–producing nontyphoid *Salmonella* with reduced susceptibility to ciprofloxacin among food animals and humans in Korea. J Clin Microbiol 2011;49:2671–2675.

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