Identification of transferable DHA-1 type AmpC $\beta$-lactamases and two mutations in quinolone resistance-determining regions of Salmonella enterica serovar Thompson

Salmonella species are important enteric pathogens causing a variety of human illnesses, especially gastroenteritis, within the paediatric population (Chyou et al., 1988). In the USA, Salmonella enterica causes approximately 1.4 million cases of gastroenteritis per year (Mead et al., 1999). Human enteric infections caused by Salmonella species are usually due to foodborne transmission and exposure to animals or animal products (Winokur et al., 2000). Antimicrobial agents used for the treatment of invasive infections caused by S. enterica include extended-spectrum cephalosporins and fluoroquinolones. Resistance of Salmonella species to extended-spectrum cephalosporins is often due to the production of various plasmid-borne $\beta$-lactamases, including extended-spectrum $\beta$-lactamases (ESBLs) and AmpC $\beta$-lactamases (González-Sanz et al., 2009). CTX-M-type ESBLs have become the main cause of resistance to third-generation cephalosporins among species of Salmonella (González-Sanz et al., 2009).

Plasmid-borne AmpC $\beta$-lactamases are strongly associated with cephalosporin resistance in Salmonella species (González-Sanz et al., 2009). Resistance to fluoroquinolones mainly results from chromosomal alterations in the quinolone-resistance-determining regions (QDRRs) of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE), and from the acquisition of plasmid-mediated quinolone resistance (PMQR) determinants, including QepA efflux, QNR proteins and AAC-(6′)-Ib-cr (Cattoir & Nordmann, 2009). Although multidrug resistance to antimicrobials is found commonly among non-typhoidal S. enterica isolates, especially S. enterica serovar Typhimurium isolates (Cui et al., 2009; González-Sanz et al., 2009; Yu et al., 2011b), resistance determinants for S. enterica serovar Thompson isolates are not well-characterized.

In September 2007, S. enterica Thompson NC24 was isolated from the stool sample of a paediatric inpatient (3 years of age) with diarrhoea in Jiangxi Provincial Paediatric Hospital, Nanchang, China. The strain was identified as S. enterica Thompson using standard biochemical tests and commercial typing antisera in accordance with the manufacturer’s instructions (Chengdu Biotech) and the serotype was assigned according to the Kauffmann–White scheme. The patient was successfully treated with intravenous ceftizoxime and was discharged after 5 days. From May 2007 to March 2009, in Jiangxi Provincial Paediatric Hospital, a total of 72 non-typhoidal S. enterica isolates representing eight distinct serotypes were recovered from stool samples collected from paediatric inpatients with diarrhoea. Although more commonly isolated from poultry, S. enterica Thompson may cause human infection via food-borne transmission. Two outbreaks of S. enterica Thompson infection resulting from contaminated food have been reported in the USA (Campbell et al., 2001; Kimura et al., 2005). Another outbreak of S. enterica Thompson infection was linked to imported rucoila lettuce in Norway (Nygaard et al., 2008). S. enterica Thompson was also the sixth most frequently isolated Salmonella species in one 10-year study performed in Canada (Khakhria et al., 1997). However, of the 72 non-typhoidal S. enterica strains isolated from May 2007 to March 2009 in Jiangxi Provincial Paediatric Hospital, only two isolates were identified as S. enterica Thompson, accounting for 2.8% of the isolates. In previous studies, it was reported that S. enterica Thompson is not frequently recovered in the context of human infection in China (Cui et al., 2009; Xia et al., 2009). In a study conducted in Wuhan, China, 29 distinct serotypes were identified among 221 S. enterica isolates collected from paediatric outpatient stool samples but S. enterica Thompson was not recovered (Cui et al., 2009).

Antimicrobial susceptibility testing of the isolate was performed using the disk diffusion method with antimicrobial agents in accordance with the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2008). The antimicrobial agents tested included (μg per disk) ampicillin (10), cefaclor (30), cefotaxime (30), ceftazidime (30), aztreonam (30), cefoxitin (30), piperacillin–tazobactam (100/10), imipenem (10), meropenem (10), chloramphenicol (30), tetracycline (30), trimethoprim/sulphamethoxazole (1.25/23.75), amikacin (30), tobramycin (10), gentamicin (10), nalidixic acid (30), ciprofloxacin (5) and levofloxacin (5). All disks were obtained from Oxoid. Mean MICs for cefotaxime, ceftazidime, cefoxitin, ciprofloxacin and levofloxacin were determined using the agar dilution method according to the CLSI guidelines (CLSI, 2008). Escherichia coli ATCC 25922 was used as quality control strain for antimicrobial susceptibility testing. S. enterica Thompson NC24 was resistant to 12 antimicrobials tested, including ampicillin, ampicillin/sulbactam, cefaclor, ceftazidime, cefoxitin, chloramphenicol, trimethoprim/sulphamethoxazole, gentamicin, tobramycin, nalidixic acid, ciprofloxacin and levofloxacin. The isolate demonstrated intermediate-level resistance to cefotaxime, piperacillin–tazobactam, aztreonam and amikacin and susceptibility to tetracycline, imipenem and meropenem. The MICs of ceftazidime, cefoxitin, ciprofloxacin and levofloxacin were 128, 16, 128, 8 and 8 μg ml$^{-1}$, respectively. However, a study conducted in Henan province, China reported that S. enterica Thompson accounted for 2.9% of all non-typhoid Salmonella isolates collected over a 1-year period, and these
isolates were susceptible to all antimicrobials tested (Xia et al., 2009).

AmpCs expressed by the isolate were identified by using the three-dimensional extract method (Coudron et al., 2000). Family-specific plasmid-mediated AmpC β-lactamase genes including acc, cit, dha, ebc, fox and mox were detected using a multiplex PCR protocol as described previously (Pérez-Pérez & Hanson, 2002). PCRs were performed for the detection of ESBL genes, including blaTEM, blaSHV and blaCTX-M, using previously described oligonucleotide primers (Yu et al., 2007). The isolate was positive for blaTEM detected by PCR, and DNA sequencing confirmed the identity of the blaTEM amplicon as blaTEM-1b. The blaVIM-1 gene was also identified using multiplex PCR and DNA sequencing. Resistance of the isolate to cephalosporins, including cefoxitin, was likely to be the result of DHA-1-type AmpC production. Although blaVIM-1 and blaCMY-2 genes have been identified previously in Salmonella isolates (González-Sanz et al., 2009), to our knowledge, this is the first report describing the existence of plasmid-borne AmpC genes in S. enterica Thompson. The isolate did not harbour blaSHV or blaCTX-M genes and did not express ESBLs as determined by the CLSI-recommended confirmatory double-disc combination test (CLSI, 2008). However, for S. enterica serovar Typhimurium isolates obtained from hospitalized paediatric patients with diarrhoea in the same hospital, broad-spectrum cephalosporin resistance was associated with production of CTX-M-type ESBLs (Yu et al., 2011b).

Point mutations in QDRs for gyrA, gyrB, parC and parE genes were determined by PCR and DNA sequencing as described previously (Giraud et al., 1999). PCR was also used to amplify PMQR determinants including the genes qnrA, qnrB, qnrS, qepA and aac(6′)-Ib-cr as described previously (Kim et al., 2009). Mutations in QDRs for GyrA and ParC were identified in the isolate at codon 83(S83F) and codon 80(S80R), respectively. PMQR determinants including the genes qnrA, qnrB, qnrS, qepA and aac(6′)-Ib-cr were not detected by PCR in the isolate. Although our patient had not received fluoroquinolones prior to isolation of the organism, the isolate exhibited resistance to nalidixic acid, ciprofloxacin and levofloxacin. In our previous study, a high prevalence of PMQR gene aac(6′)-Ib-cr associated with low-level quinolone resistance was found among S. enterica Typhimurium isolates from hospitalized paediatric patients with diarrhoea (Yu et al., 2011a). It is of note that another S. enterica Thompson isolate, NC36, collected more recently from the same hospital, also harboured resistance to multiple antimicrobials, including third-generation cephalosporins and cefoxitin (data not shown). However, S. enterica Thompson NC36 did not harbour blaVIM-1 and blaTEM-1 genes and mutations in QDRs were not found in the isolate (data not shown), indicating that additional resistance mechanisms may contribute to the multidrug resistance of S. enterica Thompson NC36. The cause of this multidrug resistance in S. enterica Thompson NC36 deserves further investigation.

In order to determine whether cephalosporin resistance in the S. enterica Thompson NC24 isolate was transferable, a conjugal transfer experiment was carried out in Luria–Bertani broth with E. coli J53 as the recipient. Cephalosporin resistance in the isolate was transferable by conjugation to recipient E. coli J53 with a frequency of $10^{-2}$. The transconjugant was resistant to ampicillin, cefaclor, ampicillin/sulbactam, ceftazidime, cefoxitin, chloramphenicol, trimethoprim/sulfamethoxazole, gentamicin and tobramycin. The MICs of ceftazidime, cefotaxime, cefoxitin, ciprofloxacin and levofloxacin for the transconjugant were 64, 16, 64, 0.25 and 0.25 μg ml⁻¹, respectively. PCR confirmed that the transconjugant harbour the blaVIM-1 gene, encoding AmpC β-lactamases, and the blaTEM-1b gene, encoding narrow-spectrum β-lactamases.

In conclusion, transmissible DHA-1 type AmpC β-lactamase production contributed to the S. enterica Thompson NC24 isolate’s resistance to broad-spectrum cephalosporins and cefoxitin. Furthermore, resistance of the isolate to fluoroquinolones was associated with mutations in the gyrA and parC genes. The presence and transmissible nature of multidrug resistance in S. enterica Thompson NC24 is of concern and suggests that even if this pathogen causes only a small proportion of clinical infections attributed to Salmonella species, its presence may contribute to resistance in other organisms that are more common human pathogens. Our findings suggest that there is a need for more aggressive surveillance to document the antibiotic resistance patterns of Salmonella isolates from paediatric patients to better inform epidemiologists as to the source of multidrug resistant organisms so as to support efforts to limit the unnecessary use of antibiotics to treat mild paediatric illnesses and poultry intended for human consumption.

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