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Prevalence and characterization of *Salmonella enterica* in dried milk-related infant foods in Shaanxi, China

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ABSTRACT

The aim of this study was to investigate the existence and characteristics of *Salmonella enterica* in dried milk-related infant foods. Twenty-four (3.4%) of 705 samples, including 5 (2.0%) of 246 powdered infant formula, 18 (4.0%) of 445 infant rice cereal, and 1 (7.1%) of 14 other infant foods, were positive for *Salmonella*. Fifteen serotypes were identified in 40 *Salmonella* isolates; *Salmonella* Duesseldorf (15.0%) and *Salmonella* Indiana (15.0%) were more frequently detected than other serotypes. Resistance to chloramphenicol (82.5%) was most common, followed by tetracycline (57.5%), ceftiofur (52.5%), kanamycin (52.5%), streptomycin (50.0%), gentamycin (45.0%), nalidixic acid (35.0%), ceftriaxone (32.5%), ciprofloxacin (25.0%), amikacin (20.0%), and cefoxitin (15.0%). Twenty-eight (70.0%) isolates were resistant to ≥8 antimicrobials, with 5 (12.5%) being resistant to 14 antimicrobials. Amino acid substitutions in gyrase A (GyrA) were most frequently detected as Ser83Arg/Asp87Glu and in p53-associated Parkin-like cytoplasmic protein (ParC), they were all Ser80Arg; the quinolone resistance gene *qnrS* (47.5%) was commonly detected as well as aminoglycoside acetyltransferase [*aac(6′)-Ib*; 25.0%], *qnrA* (17.5%), and *qnrB* (15.0%) genes. Thirty distinct pulsed-field gel electrophoresis patterns were identified among 40 isolates; no identical pulsed-field gel electrophoresis pattern was detected among *Salmonella* isolates with the same serovar that was recovered in 2010 and 2012. Our results suggest that dried milk-related infant foods could be contaminated with *Salmonella* and highlight that the dangers to infant health should not be neglected.

Key words: *Salmonella*, infant food, antimicrobial resistance, pulsed-field gel electrophoresis

INTRODUCTION

Dried infant foods, including powdered infant formula (**PIF**), infant rice cereal (**IRC**), and other infant foods (**OIF**) are not sterile products and may be intrinsically contaminated with pathogens, such as *Salmonella enterica* and *Cronobacter sakazakii*, which can result in serious illness among infants (i.e., children aged <1 yr; FAO/WHO, 2004). Although *C. sakazakii* has become an infant food safety issue due to the severity of disease it may cause in infants, concerns regarding *S. enterica* in milk-related infant foods have been somewhat overshadowed (Iversen and Forsythe, 2004; FAO/WHO, 2006).

Currently, the tolerance limits for *Salmonella* in milk and milk-related products in Canada, Cuba, Chile, European Union, the Netherlands, Australia, New Zealand, China, Singapore, and South Africa are all 0 cfu/25 g (mL) of sample, which means no *Salmonella* should be detected in milk, PIF, cheese, or yogurt (ICMSF, 1998; Wu et al., 2006). Although efforts have been made to control *Salmonella* in different milk-related products, in the 20-yr period from 1985 to 2005, at least 6 outbreaks of *Salmonella* infection have occurred in the United Kingdom, the United States, Canada, Spain, France, and Korea, associated with consumption of PIF, involving approximate 287 infants (Rowe et al., 1987; Centers for Disease Control and Prevention, 1993; Usera et al., 1996; Threlfall et al., 1998; Park et al., 2004; Brouard et al., 2007). The common characteristic of these outbreaks was the low level of *Salmonella* detected in the implicated PIF; thus, *Salmonella* may be ignored or missed during routine testing. The outbreaks likely represent only a tiny proportion of the actual number of *Salmonella* infections among infants that had been involved in PIF and OIF (Cahill et al., 2008).

In this study, the prevalence and characterization of *Salmonella* isolates recovered from dried milk-related infant foods were investigated in Shaanxi Province (China) to provide meaningful data for better understanding of infant food safety.

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MATERIALS AND METHODS

Sample Collection and Salmonella Isolation

A total of 705 dried milk-related infant foods, including 445 IRC, 246 PIF, and 14 OIF samples covering 71 brands, were randomly collected from 51 supermarkets and 32 retail stores in 12 cities (Xi'an, Yangling, Baoji, Meixian, Qishan, Qianxian, Xingping, Fufeng, Liquan, Zhouzhi, Wugong, and Xianyang) in Shaanxi Province (China) in 2010 and 2012. Two to 5 representative supermarkets and retail stores were selected in each city for sampling. In each supermarket/store, specific brands of IRC and PIF, including imported and domestically produced ones, were sampled.

Salmonella was isolated according to the standard procedures described in the National Standards of the People's Republic of China (GB 4789.4-2010). Briefly, 25-g food samples were placed in 225 mL of buffered peptone water (Beijing Land Bridge Technology Co. Ltd., Beijing, China) and incubated at 36 ± 1°C for 8 to 18 h. A 1-mL aliquot of preenriched culture was transferred to a 10-mL aliquot of tetrathionate (**TT**) broth (Beijing Land Bridge Technology Co. Ltd.) and selenite cystine (**SC**) broth (Beijing Land Bridge Technology Co. Ltd.). The TT was incubated at 42 ± 1°C and SC at 36 ± 1°C for 18 to 24 h. One loop of TT broth was streaked onto bismuth sulfite (**BS**; Beijing Land Bridge Technology Co. Ltd.) and SC broth onto Hektoen enteric (**HE**; Beijing Land Bridge Technology Co. Ltd.) agars. The BS plates were incubated at 36 ± 1°C for 40 to 48 h and HE plates for 18 to 24 h, and examined for the presence of *Salmonella*. The presumptive isolates on BS and HE plates were subcultured on Luria-Bertani agar plates (Beijing Land Bridge Technology Co. Ltd.) at 36 ± 1°C from 18 to 24 h, and 1 colony on Luria-Bertani plate was selected and confirmed with API20E identification kits (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions.

Serotyping

The O and H antigens of *Salmonella* isolates were characterized with hyperimmune sera (S&A Reagents Lab Ltd., Bangkok, Thailand; Statens Serum Institut, Copenhagen, Denmark). The serotype was identified using the slide agglutination method and assigned following the manufacturer's instructions and the Kauffmann-White classification scheme.

Antimicrobial Susceptibility Testing

Antimicrobial MIC were determined by an agar dilution method using Mueller-Hinton agar (Beijing Land Bridge Technology Co. Ltd.) according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2010). The antibiotics amikacin, gentamycin, kanamycin, streptomycin (**STR**), ampicillin, amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, cefoxitin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin (**CIP**), nalidixic acid (**NAL**), and tetracycline were selected. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Enterococcus faecalis* ATCC 29212 were used as quality control organisms in MIC determinations. The breakpoints for interpretation of resistance and susceptibility were according to the interpretive standards of the Clinical and Laboratory Standard Institute (CLSI, 2010), except for STR, which was determined according to the breakpoints used for susceptibility testing of *Salmonella* and *Escherichia coli* of the National Antimicrobial Resistance Monitoring System (NARMS) of the United States (http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm334828.htm).

Screening for qnr and Aminoglycoside Acetyltransferase Genes, and Quinolone Resistance-Determination Region Gene Amplification and Sequencing

Salmonella isolates being resistant to NAL but susceptible or low-level resistant to CIP were examined for the presence of quinolone resistance (***qnr***) genes, including *qnrA*, *qnrB*, and *qnrS*, and the aminoglycoside acetyltransferase [***aac(6′)-Ib***] gene. Isolates that showed both NAL and CIP resistance were examined for amino acid substitutions in quinolone resistance-determination regions (**QRDR**) of gyrase A (**GyrA**) and p53-associated Parkin-like cytoplasmic protein (**ParC**). All genes were amplified using MyCycler PCR (Bio-Rad Laboratories Inc., Hercules, CA) as described previously (Park et al., 2006; Cattoir et al., 2007; Yang et al., 2012). The primers were synthesized by TaKaRa Biotechnology Co. Ltd. (Dalian, China) and are listed in Table 1. The PCR products were stained with ethidium bromide and visualized under UV light after gel electrophoresis in 1% agarose. For DNA sequence analysis, PCR products were purified with a kit (TaKaRa Agarose Gel DNA Purification Kit Ver. 2.0; TaKaRa Biotechnology Co. Ltd.), and sent for sequencing at AuGCT biotechnology Co. Ltd. (Beijing, China). The DNA sequence data were analyzed and aligned using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (**PFGE**) was carried out according to the protocol developed by the Centers