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Shigella and *Salmonella* contamination in various foodstuffs in Turkey

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Abstract

The prevalence of *Shigella* and *Salmonella* in a range of foodstuffs purchased from supermarkets and smaller units in Bursa province (Turkey) over a 7-month period between December 2004 and June 2005 was evaluated. In total 416 food samples composed from chicken parts, minced meats, ready-to-eat salads, raw vegetables and raw milks were analysed. Among the samples only one chicken thigh sample (0.24%) was found to be contaminated with *Salmonella* whereas *Shigella* was not isolated from any samples. Isolated *Salmonella* strain was serotyped as *Salmonella enterica* subsp. *enterica* serovar Infantis (S. Infantis) and displayed multidrug resistance to several antibiotics including streptomycin, tetracycline, sulphonamides, trimethoprim, trimethoprim-sulphamethoxazole and nalidixic acid. Decreased susceptibility to ciprofloxacin (MIC 0.38 mg/L by *E*-test) was also determined. The present study revealed that despite low contamination rate, foodstuffs particularly chicken parts could be a potential vehicle for foodborne infections and implementation of preventive measures and consumer food safety education efforts are needed.
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Keywords: *Shigella*; *Salmonella*; Foods; Antimicrobial susceptibility

1. Introduction

Foodborne illness is a major international health problem (Mensah, Yeboah-Manu, Owusu-Darko, & Ablordey, 2002). Each year, millions of persons become ill from foodborne diseases, though many cases are not reported (CDC, 1997). Shigellosis, also called bacillary dysentery, is an infectious disease caused by *Shigella* bacteria (Mead et al., 1999). The species involved are mostly *Shigella sonnei* (about 70%) and *S. flexneri* (approximately 25%) while other species are seldomly implicated (Uyttendaele, Bagamboula, De Smet, Van Wilder, & Debevere, 2001). *Shigella* species have the potential to cause large outbreaks because of their low infectious dose (~10 cells). Foods and drinking waters can serve as vehicles of transmission of this pathogen (DuPont, Levine, Hornick, & Formal, 1989; Wu et al., 2000). Each year, there are significant numbers of shigellosis outbreaks resulted from consumption of contaminated foods (Mead et al., 1999).

Salmonella is one of the most important pathogenic genera implicated in foodborne bacterial outbreaks and diseases (Gouws, Visser, & Brözel, 1998). *Salmonella* infections are worldwide and constitute an important public health problem in Turkey as well as in many parts of the world (Erdem, Ercis, Hascelik, Gur, & Aysev, 2005). It was reported that *Salmonella* causes an estimated 1.4 million cases of foodborne illness and more than 500 deaths per year in the US (CDC). There are several transmission routes for salmonellosis, but the majority of human infections are derived from the consumption of contaminated foods especially those of animal origin (Hernandez et al., 2005). A variety of food products, especially poultry and other types of meat products, are the most important sources of human *Salmonella* infection (Chittick, Sulka, Tauxe, & Fry, 2006; Jay, 2000; Orji, Onuigbo, & Mbata,

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2005). International or multistate outbreaks caused by a range of foodstuffs contaminated with different *Salmonella* serotypes have been reported (Buchholz et al., 2005; Holtby, Tebbutt, Harrison, & Kett, 1995; McLaughlin, Castrodale, Gardner, Ahmed, & Gessner, 2006).

In recent years, another health concern all over the world is the occurrence of antibiotic resistant strains of a number of pathogenic bacteria including *Salmonella,* in foods (Busani et al., 2004). The extensive use of antibiotics for therapeutic or preventive purposes in veterinary medicine and as growth promoters in animal feed has contributed to the occurrence of resistant bacteria in animals, including zoonotic pathogens, which can be transmitted to humans via food chain (Su, Chiu, Chu, & Ou, 2004; Tollefson & Miller, 2000).

This study was conducted to determine the incidence of *Shigella* and *Salmonella* in retail foodstuffs and raw milks in Bursa (Turkey), and to characterize the isolates for their serotypes and antibiotic resistance.

2. Materials and methods

2.1. Sample collection

From December 2004 to June 2005, a total of 416 food samples were collected in Bursa province, Turkey. Samples included were: (a) chicken parts: thighs, drumsticks, breasts, wings, necks of broiler chickens ($n = 168$), (b) minced meats ($n = 45$), (c) ready-to-eat salads (containing raw vegetables, tuna, mayonnaise, potato, chicken) ($n = 100$), (d) raw vegetables generally consumed in raw form (lettuce, spinach, rocket, dill, parsley, green onion, green garlic, carrot, red cabbage) ($n = 78$), and (e) raw cow's milks ($n = 25$). Chicken parts and minced meats were purchased from large supermarkets and smaller units including butcher shops and poulterer shops; ready-to-eat salads were from supermarkets, restaurants and fast-food centres; and raw vegetables were from supermarkets and neighbourhood bazaars. Raw milks were provided from several dairies. All samples were taken by using sterilized utensils and placed in separate sterile plastic bags but glass bottles were used for raw milks. Samples were transported to the laboratory immediately after sampling. Bacteriological analyses were carried out on the day of their arrival at laboratory.

2.2. Microbiological analysis

For isolation of *Shigella* spp., conventional culture method described by Food and Drug Administration (FDA) was used (Andrews & Jacobson, 2000). Samples (25 g or ml) were homogenized by means of a seward stomacher, model 400 circulator ($\sim$ 2 min) in 225 ml shigella broth in which novobiocin (Sigma) was added (0.5 μg/ml for *S. sonnei* and 3.0 μg/ml for other *Shigella* spp.). It should be noted that, for chicken samples 25 g sample was taken from skin and muscle tissues of multiple points on chicken parts. The homogenized samples were then anaerobically incubated for 20 h at 44 °C and 42 °C for *S. sonnei* and other *Shigella* species, respectively. Following incubations, a loopful from each of enrichment cultures was streaked onto MacConkey (Oxoid, CM0007) and SS (Oxoid, CM0099) agar plates. The plates were incubated for 20 h at 37 °C before assessment for the presence of characteristic presumptive *Shigella* colonies (slightly pink and translucent on MacConkey and colorless, non-lactose fermenting on SS agar). The presumptive isolates were streaked and stabbed into tubed slants of triple sugar iron agar (Merck, 1.03915.0500). After 24 to 48 h incubation at 37 °C, motility-, H_2S- and glucose (gas)-negative isolates were screened for urease, indole and ONPG by using the HY-Enterotest (TT 146, Hy Laboratories Ltd., Israel) system.

For isolation of *Salmonella* spp., 25 g of each sample was homogenized in 225 ml of lactose broth and incubated at 37 °C overnight. One milliliter of this pre-enrichment culture was inoculated into tubes containing 10 ml tetrathionate (TT) broth (Oxoid, CM0029) for enrichment, and incubated 24 h at 37 °C. Bacteria growing in TT broth were streaked on xylose lysine desoxycholate (XLD) agar (Oxoid, CM0469) and MacConkey agar (Oxoid, CM0007) and incubated at 37 °C overnight. The plates were examined for the presence of typical colonies of *Salmonella*, i.e. pink colonies with or without black centres on XLD agar and colourless colonies on MacConkey agar. Presumptive *Salmonella* colonies were then subjected to initial screening tests using triple sugar iron agar, lysine iron agar (Merck, 1.11640.0500), urea broth (Merck, 1.08483.0500) and lysine decarboxylase broth (Oxoid, CM308). All biochemical tests were performed at 37 °C for 18–24 h (Andrews & Hammack, 1998). Presumptive positive colonies were subjected to Poly O (Denka Seiken, Tokyo, Japan) and Poly H (Denka Seiken) slide agglutination tests. *Salmonella* serotyping was performed according to the Kauffmann–White scheme (Popoff, 2001) at Enteric Pathogens Reference Laboratory of Refik Saydam National Hygiene Center, Ankara, Turkey.

2.3. Assessment of antimicrobial susceptibility

Antimicrobial susceptibility tests were carried out by the disc diffusion technique described by Kirby–Bauer on Mueller–Hinton agar (Oxoid, Basingstoke, Hampshire, UK). Antimicrobials used, and their concentrations are as follows: ampicillin (A; 10 μg), amoxicillin/clavulanic acid (Amc; 20/10 μg), chloramphenicol (C; 30 μg), gentamicin (G; 10 μg), kanamycin (K; 30 μg), streptomycin (S; 10 μg), sulphonamides (Su; 300 μg), tetracycline (T; 30 μg), trimethoprim (Tm; 5 μg), trimethoprim/sulfamethoxazole (Sxt; 1.25/23.75 μg), ciprofloxacin (Cp; 5 μg), nalidixic acid (Nx; 30 μg), amikacin (Ak; 30 μg), cephalothin (Ch; 30 μg), cefotaxime (Ctx; 30 μg), ceftizoxime (Ctz; 30 μg), and ceftriaxone (Cr; 30 μg) (Oxoid, Basingstoke, UK). Results were recorded by measuring the inhibition zones and scored as susceptible or resistant according to

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